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EFFECT OF PHOSPHORUS REMOVAL PROCESSES ON ALGAL GROWTH

Jan Scherfig, et al

California University

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EFFECT OF PHOSPHORUS REMOVAL PROCESSES ON ALGAL GROWTH

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ABSTRACT

Laboratory studies were conducted to improve algal assay techniques for use in evaluation of sewage treatment processes.

Laboratory studies (batch and continuous cultures) were conducted at the Santee, California water reclamation plant to evaluate the effect of tertiary waste treatment processes on the amount of algal growth in the treated effluent.

Laboratory studies were also conducted to determine the growth limiting nutrients in each type of tertiary effluent.

Field tests were conducted using special study ponds and the results of the field tests were compared with the laboratory test results. The laboratory and field tests showed the same relative ranking for the treated effluents.

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[27] Abstract

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SECTION I

CONCLUSIONS

1. This research project showed that algal assays are very useful tools for evaluating waste treatment processes.

2. The batch algal assay can be used as a routine method at this time.

3. Continuous culture assays of very highly treated effluents such as those resulting from electrodialysis processes are not yet practical on a routine basis.

4. The results of the batch algal assays indicate that the nutrient which limits growth of algae changes as a result of treatment. Thus, it is very important in treatment plant evaluations to conduct parallel algal assays and determine the actual limiting nutrient or nutrients after each process step.

5. The batch algal assays performed in this study showed that phosphate was not the only nutrient limiting growth of the test algae under laboratory conditions. The results indicated that other nutrients especially iron played a significant role in limiting algal growth.

6. Based on the laboratory results, it appears that the question of growth limiting nutrients is very complex and that the concept of a simple limiting nutrient is not valid. Instead, there appears to be several strong positive and negative interactions among nutrients such as nitrogen, phosphorus, iron, manganese, and some of the major cations and anions. It appears necessary to provide further insight into these interactions before a more rational approach to design of nutrient removal processes can be made.

7. On a gross biomass basis, it was found that batch and continuous culture laboratory assays provided the same ranking of effluents from different treatment processes.

8. Using Santee secondary effluent as the basis, the continuous culture algal investigations in the laboratory showed that the lime treatment plus electrodialysis resulted in a 100 fold reduction in the algal growth.

9. Comparison of electrodialysis and ground percolation indicated that at Santee the two processes were approximately equally effective in reducing algal growth as measured by the batch assay test.

10. Two study ponds were built and used to compare the secondary and tertiary effluents. It was found that when the two types of effluent were exposed to the same environment, the tertiary effluent (lime treatment) resulted in less algal growth.

11. The changes in agal growth in the study ponds due to changes in natural conditions such as light and temperature were greater than the difference in growth of algae due to the difference in quality between secondary and tertiary effluents. It is concluded that in future studies, study ponds should preferably be operated over two full years to obtain statistically better results.

SECTION II

RECOMMENDATIONS

1. Evaluation of tertiary treatment processes should include algal assays in addition to routine chemical effluent analyses.

2. Additional work should be done to understand better the nature of the interactions occurring between different nutrients in treated effluents.

3. Much additional work should be conducted on the relationship between the laboratory assay results and the growth of natural algal populations in small study ponds. This will serve as a step towards understanding the effect of testing effluents on large natural or artificial bodies of water.

SECTION III

INTRODUCTION

Increasing eutrophication of surface water combined with a growing need for recreational water in arrid areas necessitates greater concern for the removal of biostimulants including the classic nutrients from treated wastewater. In the past, the major emphasis has been placed on methods for reducing the phosphorous content in effluents by combinations of chemical and biological processes. However, there is little scientific evidence indicating that the efficiency of a treatment process in terms of the removal of biostimulants, can be measured only by the degree of phosphorous removal. Other nutrients may also be removed by the complex and not well understood biological and chemical phenomena occuring in phosphate removal processes. On the other hand, organic compounds acting as growth stimulants may be produced in significant quantities in traditional biological waste treatment processes. Either of these phenomena can be expected to produce a significant change in algal growth rates and yields when compared to phosphate removal alone.

Because of these complex and little understood phenomena there is a need for a better method for evaluating the efficiency of both existing and proposed phosphate removal processes with respect to the removal of other biostimulants. Similarly there is a great need for a better understanding of the relative efficiencies of individual steps in a sequence of treatment processes; to be meaningful, the efficiencies must be measured in terms of actual reduced algal growth. Such information can provide a more rational basis for the optimum design of wastewater reclamation facilities.

The research program consisted of two overlapping parts; the development of batch and continuous algal assay procedures as well as the field evaluation of specific treatment processes.

The development of algal assay procedures was conducted in close cooperation with several other groups of investigators coordinated under the National Eutrophication Research Program, Pacific Northwest Water Laboratory, Corvallis (U. S. Environmental Protection Agency).

The field evaluation of treatment methods for the removal of algal nutrients was conducted in cooperation with the Santee County Water District, which was conducting a demonstration project under partial sponsorship of the U.S. Environmental Protection Agency (Grant #WPRD5-01-67). The field evaluation included comparison of algal growth in secondary effluents, tertiary effluants, effluent from an electrodialysis process and effluent from a ground percolation process. The laboratory evaluations were supplemented by evaluation of the effluents in large scale study ponds located at the site of the Santee County Water District's demonstration facility.

SECTION IV

BATCH CULTURE ASSAY METHODS

Throughout the entire project sample preparation and test procedures were under continuous development and evaluation. This resulted in a series of changes in the detailed techniques used.

The evaluation of procedures were conducted in close coordination with other laboratories doing related research. The evaluation of procedures was coordinated by the Pacific Northwest Water Laboratory of the U.S. Environmental Protection Agency. The results obtained in the work described here have been incorporated in the Algal Assay Procedure Bottle Test (Reference IV-1) and no additional recommendations are made with regard to the Bottle Test.

The specific aspects of the test development which was conducted at UCI included:

1. Medium Preparation Procedure
2. Effect of CO_2 Addition
3. Effect of Light Intensity
4. Method of Air Addition
5. Role of Medium Concentration
6. Glassware Preparation
7. Sterilization Techniques
8. Evaluation of Materials for Algal Culture Systems

INITIAL METHODS

A culture of the green alga Selenastrum capricornutum Printz was obtained from the National Eutrophication Research Program; Pacific Northwest Water Laboratory; Corvallis, Oregon, 97330. This species is one of three algae selected as standard organisms for use in the Provisional Algal Assay Procedures. Cultures were grown at a temperature of 24°C± 2°C in one liter Pyrex Erlenmeyer flasks fitted with a neoprene rubber stopper and 1/8" I.D. Pyrex tube in- and outlets, through which all gases were added. All glassware and culture vessel components were acid cleaned and autoclaved according to Provisional Algal Assay Procedures.

Air or a "CO_2-enriched" air mixture was pumped continuously into each flask and either bubbled through the medium ("aerated") or passed over the surface of the medium ("ventilated") at a flow rate of 150-200 ml/min. Carbon dioxide

was supplied from a 100% CO_2 compressed gas cylinder and released at low pressure into a special gas mixing chamber (Matheson Model 665) by means of a dual regulator system (Matheson Model 9 and Matheson Model 70A). Air was provided by "Silent Giant" air pumps (Aquarium Pump Supply, Inc.) to achieve the specified flow rate in each test flask. The concentration of CO_2 was controlled by a needle valve at the CO_2 entrance into the gas mixing chamber. All gases were humidified by bubbling through distilled water to stimulate the effects of evaporation and then sterilized by passing through a Millipore filter (0.45μ) in a special Millipore filter holder (Millipore #YY30 142 00). The concentration of carbon dioxide was varied throught the period of experimentation so as to maintain the pH between 7.0 and 8.0 in all CO_2 receiving cultures. Each day pH measurements were taken and the necessary CO_2 adjustments made. Mixing was accomplished by a 2" x 3/8" Teflon spin bar driven by a magnetic stirrer at 300 $\pm$ 50

Cultures were continuously illuminated by Sears 40W "Cool White" Fluorescent lamps. Aluminum foil screens were used to ensure the selected light intensity at the midpoint of each flask. Intensity was measured by a Weston illumination meter (Model 703) and expressed as foot candles. The experimental arrangement is shown in Figure 1.

Flasks were inoculated at the beginning of each experiment with a culture of Selenastrum to a concentration of 10^3 cells/ml. Cells for inoculation were separated by centrifugation from an actively growing culture (400 foot candles, modified PAAP Medium) In order to minimize possible effects of nutrient carryover, the cells were rinsed in a special wash solution with a composition as indicated in Table 1, and then resuspended for 48 hours in fresh wash solution prior to inoculation. The wash solution containing the algal cells was then added volumetrically (3-5 ml) so as to produce the prescribed number of cells in each test flask.

Algal growth was measured gravimetrically by the vacuum filtration of a measured portion of algal suspension through a preweighted Millipore filter (0.45μ) and expressed as dry weight. The methods of handling, drying and weighing the filters were essentially those outlined in the Provisional Algal Assay Procedures. Weights were measured on the fifth or sixth day after inoculation and every other day thereafter until the seventeenth or eighteenth day. In experiments where cultures were still actively growing on the eighteenth day, as in the multiple concentrated medium tests, measurements were continued every five to seven days until the experiment was terminated.

INVESTIGATION OF MEDIUM PREPARATION PROCEDURE

The culture medium as outlined in the Provisional Algal Assay Procedures was found initially to give relatively low algal growth rates and yields. It appeared that trace metals were removed during filter sterilization, and that the amounts removed varied from time to time. The method of preparation was therefore modified (Table 2) and the effects of this modification determined. Six replicates were prepared individually using both the procedure outlined in the Provisional Algal Assay Procedures and the modified procedure. 800 mℓ of each medium (within the theoretical composition shown in Table 3) was added to each of six one liter flasks and the relative algal growth rates and yields determined. Cultures were inoculated as previously described, grown at 400 foot candles and "aerated" with CO_2-enriched" air mixture (0-2%) bubbled through the culture in sufficient quantity to maintain the pH of each algal culture between 7.0 and 8.0 throughout the period of experimentation. The resulting growth of Selenastrum in the unmodified and the modified medium is shown in Table 4 a and Figure 2.

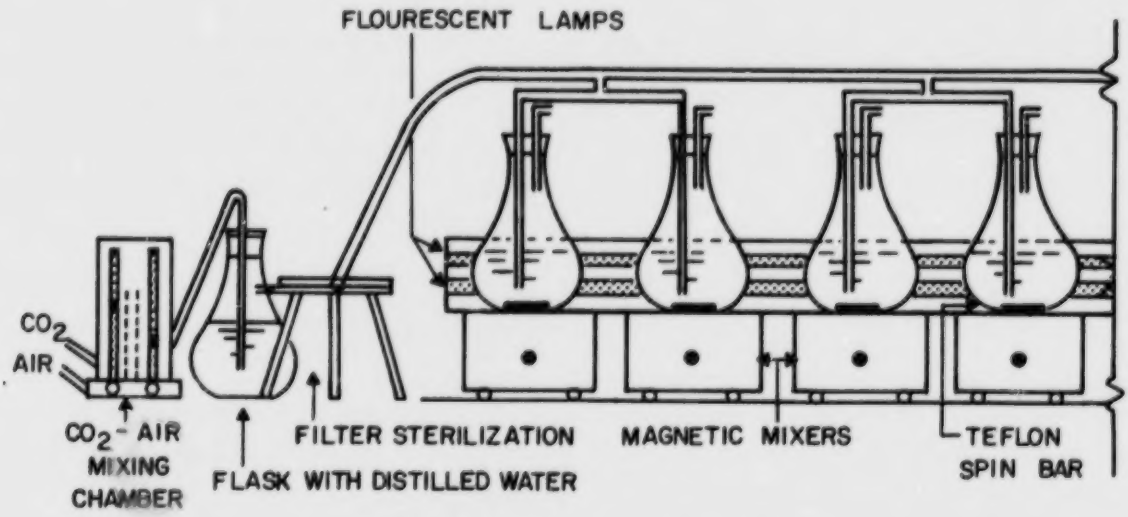

FIGURE 1. INITIAL EXPERIMENTAL BATCH CULTURE SYSTEM

$MgCl_2.6H_2O$	41.20 mg/ℓ
$MgSO_4$	23.81 mg/ℓ
$CaCl_2$	11.32 mg/ℓ

TABLE 1. WASH SOLUTION COMPOSITION

TABLE 2. INITIAL MEDIUM PREPARATION PROCEDURES

Unmodified PAAP Procedure	Modified PAAP Procedure
1. Prepare 1 liter stock solutions of individual salts at 1000 times concentration.	1. $FeCl_3.6H_2O$ and Na_2CO_3 are not prepared as stock solutions but instead are weighed and added directly at each preparation.
2. Prepare a 1 liter combined trace metal and $Na_2EDTA.2H_2O$ stock solutions at 1000 times concentration.	2. H_3BO_3, $MnCl_2.4H_2O$, $ZnCl_2$, $CoCl_2 6H_2O$, $CuCl_2$, $NaMoO_4.2H_2O$ and $Na_2EDTA.\ 2H_2O$ are each prepared as individual 1 liter stock stock solutions at 1000 times concentration.
3. Add 1 ml of each stock solutions to enough glass distilled water to make 1 liter of culture medium, adding K_2HPO_4 last.	3. Add 1 ml per ℓ of each stock solution to make glass distilled water to make 1 ℓ in the following order, adjusting the pH where indicated: $NaNO_3$, $MgCl_2.6H_2O$, $MgSO_4$, $CaCl_2$, Add 50 mg/ℓ of Na_2CO_3 Adjust pH to 7.5 with HCl Add 1 ml per ℓ of $K_2HPO_4.3H_2O$ stock Add 1 ml per ℓ of each of the trace metal stock solutions and the Na_2EDTA stock solution.
4. Filter sterilize at 0.5 atm. through 0.45μ Millipore filter.	4. Filter sterilize under vacuum through 0.45μ Millipore filter. 5. Add 0.53 mg/ℓ of $FeCl_3.6H_2O$ directly from reagent bottle immediately prior to use.
Medium pH: 10.1	Medium pH: 7.4-7.6

TABLE 3. INITIAL THEORETICAL MEDIUM COMPOSITION
(Reference IV-2)

Major Salts		Trace Metal Solutions	
$NaNO_3$	85.00 mg/ℓ	H_3BO_3	618.40μg/ℓ
$K_2HPO_3.3H_2O$	4.55 mg/ℓ	$MnCl_2.4H_2O$	1384.60μg/ℓ
$MgCl_2.6H_2O$	41.20 mg/ℓ	$ZnCl_2$	109.03μg/ℓ
$MgSO_4$	23.81 mg/ℓ	$CoCl_2.6H_2O$	4.76μg/ℓ
$CaCl_2$	11.32 mg/ℓ	$CuCl_2$	0.03μg/ℓ
Na_2CO_3	50.00 mg/ℓ	$Na_2MoO_4.2H_2O$	24.20μg/ℓ
$FeCl_3.6H_2O$	0.53 mg/ℓ	$Na_2EDTA.1H_2O$	7.07 mg/ℓ

Nutrients per 1 liter glass distilled water.

TABLE 4. GROWTH OF SELENASTRUM CAPRICORNUTUM IN MODIFIED AND UNMODIFIED PAAP MEDIUM

	Unmodified Medium			Modified Medium		
Day	Mean	St. Dev.	C_v*	Mean	St. Dev.	C_v
5	.008 ±	.004	.474	.140 ±	.018	.129
9				.414 ±	.036	.087
13	.061 ±	.028	.458	.486 ±	.034	.071
17	.084 ±	.071	.845	.475 ±	.032	.067

*Coefficient of Variation (C_v) is defined as Standard Deviation divided by mean.

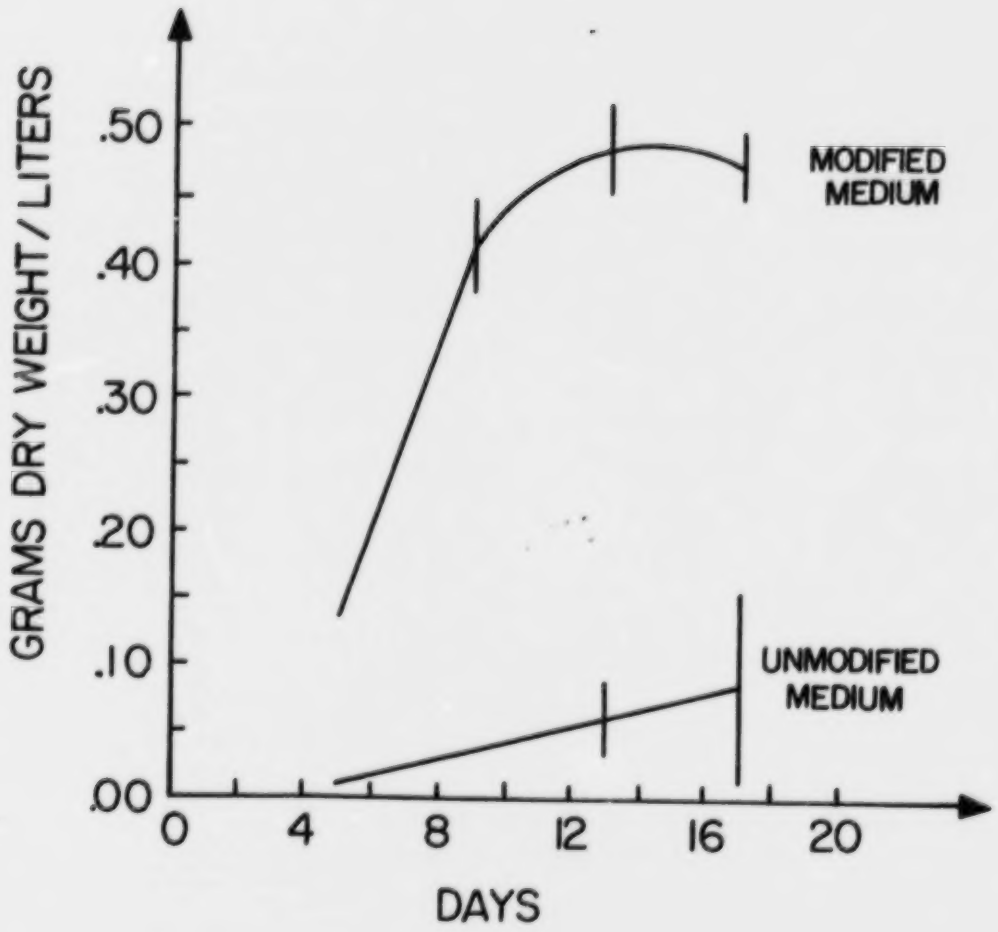

FIGURE 2. GROWTH OF SELENASTRUM CAPRICORNUTUM IN UNMODIFIED AND MODIFIED MEDIUM

Preliminary chemical analyses of the unmodified and the modified media indicated that iron and manganese had been removed from the unmodified PAAP media during filter sterilization. Consequently, an experiment was

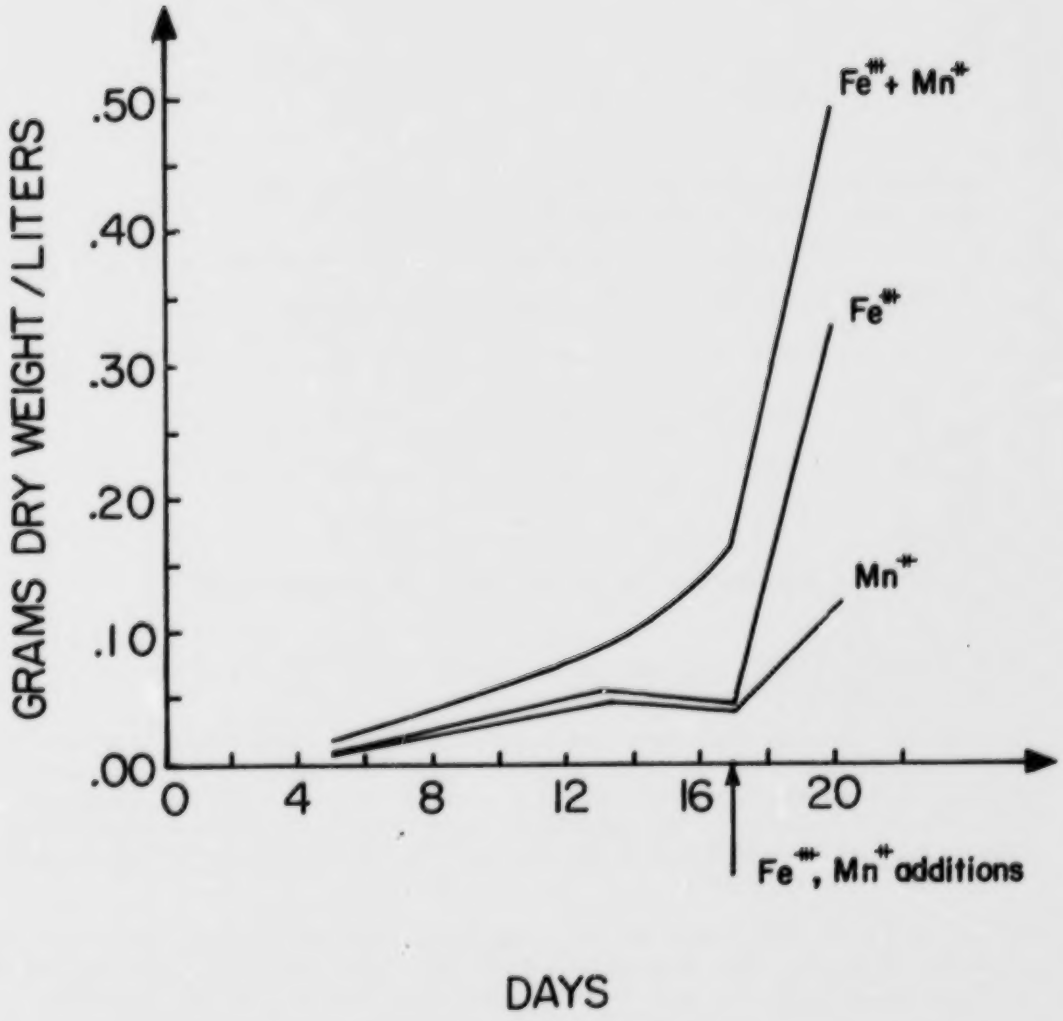

FIGURE 3. EFFECT OF IRON AND MANGANESE ADDITION TO UNMODIFIED PAAP MEDIUM

conducted in which 0.53 mg/ℓ $FeCl_3.6H_2O$ and 1.38 mg/ℓ $MnCl_2.4H_2O$ were added to the unmodified medium cultures on the seventeenth day of growth. The results of this supplementation are presented in Table 4 and Figure 3. They demonstrate a marked increase in the growth of Selenastrum immediately following the addition of iron and manganese as would be expected if iron and manganese were being removed in the medium preparation procedure.

TABLE 5. EFFECTS OF IRON AND MANGANESE ADDITION TO UNMODIFIED PAAP MEDIUM

Nutrients were added to growing cultures on the 17th day. Each value is the mean for two cultures.

Day	Fe added 17th day	Mn added 17th day	Fe, Mn added 17th day
5	.007	.007	.011
13	.052	.045	.086
17	.048	.037	.167
21	.324	.117	.465

Growth of Selenastrum capricornutum in dry weight (g/ℓ).

EFFECT OF CO_2, LIGHT, AIR AND MEDIUM CONCENTRATION

Preliminary experimental work had indicated that certain other factors were limiting both the rate of algal growth and total algal yield. The experiments presented above showed that the modified method of medium preparation minimized the effects of uncontrolled trace metal removal. Consequently, this method of medium preparation was used in the remainder of the experimental work reported here.

A 2 x 2 x 2 x 2 (2^4) factorial experiment was designed to test the effects of light intensity (350 foot candles vs. 500 foot candles), gas composition ("CO_2-enriched" air vs. non"CO_2-enriched" air), method of gas addition ("aeration" vs "ventilation") and medium concentration (100% vs 600%). The six hundred per cent concentrated medium was included in the tests to ensure that the range of algal concentrations encountered in nutrient rich samples such as secondary effluents would be covered. Two replicate experiments were run and the results are presented in Tables 6 and 7. The experiments were analyzed statistically on the basis of both the maximum volumetric growth rates (grams per liter per day) and the maximum normalized growth rates (grams per gram of algae present per day). The statistical analyses were performed with the use of the General Electric Mark II Computer Library and

the results are presented in Tables 8 and 9 respectively. All of the factors tested are shown to exert a statistically significant effect using one of the above indices of algal growth.

Maximum normalized growth rates were obtained during the earlier stages of exponential growth in all test cultures. Analyses using this index showed that the selected light intensities, methods if air addition, and "CO_2-enrichment" affected Selenastrum growth (Table 10). The results show that light at 500 foot candles decreases maximum normalized growth rates while aeration and CO_2-enrichment increases these values. Medium concentration was shown to be insignificant in affecting maximum normalized growth. This is explained by the high nutrient to cell ratio present in all cultures during the early stages of growth. Analyses also indicated the presence of an interaction between CO_2 and light intensity using maximum normalized growth rates.

Medium concentration and "CO_2-enrichment" were found to be significant factors affecting Selenastrum growth when maximum volumetric growth rates were used for analyses (Table 11). Maximum volumetric growth was found to occur during the middle to late stages of exponential growth prior to the stationary growth phase. During this period nutrient availability in the 100% concentrated medium cultures and CO_2 in the non-CO_2 enriched cultures were shown to be limiting Selenastrum growth (Table 11). An interaction between CO_2 and medium concentration was also found.

Algal yields were compared for all cultures using values obtained on the 15th and 17th-18th days of growth. Only the 100% concentrated medium cultures were used for analysis on these days as total yield figures were not obtained for the 600% concentrated medium cultures. As demonstrated by the previous analyses of growth rates, "CO_2-enrichment" was effective in accelerating Selenastrum growth on the days used for analysis, "CO_2-enriched" cultures showed greater algal yields (Table 12). Figure 4 is a composite representation of the significant factors affecting the growth of Selenastrum per unit volume in the factorial experiments.

These results suggest that nutrient availability is markedly influenced by medium pH. To test this hypothesis, medium samples from the 600% concentrated medium cultures of Selenastrum were separated from the algal cells by centrifugation at the end of the growth. Chemical analyses of the medium showed a significantly larger concentration of iron, calcium and manganese in the "CO_2-enriched" samples compared with the non "CO_2-enriched" samples, even though more algal growth had occurred in the former (Table 13). The analyses also showed greater concentrations of sodium, boron and silicon to be presented in the non"CO_2-enriched" samples (Table 13).

These findings both appear to be correlated with the effects of CO_2 on medium pH. The pH values for the"CO_2-enriched" cultures ranged from 7.5 - 8.0 at the time of analysis while the values for the non"CO_2-enriched" cultures ranged from 9.5 - 11.0.

TABLE 6. EFFECTS OF CO_2, LIGHT, AIR AND MEDIUM CONCENTRATION ON ALGAL GROWTH - REPLICATE 1

2^4 Factorial Experiment

	CO_2 Enriched Air								Non-CO_2 Enriched Air							
	350 ft-c				500 ft-c				350 ft-c				500 ft-c			
	600%		100%		600%		100%		600%		100%		600%		100%	
	Medium		Medium		Medium		Medium		Medium		Medium		Medium		Medium	
DAY	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.
6	.056	.057	.023	.067	.113	.137	.103	.063	.043	.043	.025	.025	.082	.106	.028	.070
8	.263	.278	.136	.258	.432	.514	.318	.295	.127	.185	.101	.159	.129	.234	.075	.180
10	.568	.607	.304	.403	.812	.881	.415	.430	.219	.321	.237	.320	.249	.544	.103	.296
12	.957	.984	.409	.483	1.232	1.453	.514	.489	.406	.510	.262	.418	.438	.698	.114	.408
13	1.119	1.178	.493	.497	1.514	1.711	.497	.545	.490	.556	.299	.452	.531	.798	.128	.461
15	1.530	1.545	.530	.513	1.976	2.048	.525	.532	.657	.689	.358	.503	.592	1.041	.121	.459
18	2.220	2.311	.584	.561	2.616	2.459	.590	.626	.967	.924	.418	.531	.767	1.426	.124	.494

Growth in dry weight (g/ℓ) of Selenastrum capricornutum

TABLE 7. EFFECTS OF CO_2, LIGHT, AIR AND MEDIUM CONCENTRATION ON ALGAL GROWTH - REPLICATE 2

2^4 Factorial Experiment

	CO_2 Enriched Air								Non-CO_2 Enriched Air							
	350 ft-c				500 ft-c				350 ft-c				500 ft-c			
	600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium	
DAY	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.	Vent.	Aer.
5	.043	.040	.040	.036	.084	.097	.069	.074	.032	.035	.029	.034	.069	.073	.035	.052
7	.169	.165	.132	.182	.283	.399	.245	.308	.093	.135	.055	.110	.094	.157	.058	.130
9	.388	.399	.276	.330	.554	.737	.350	.391	.117	.197	.085	.165	.123	.252	.089	.190
11	.576	.584	.363	.436	.913	1.096	.335	.496	.185	.335	.124	.238	.182	.324	.138	.265
13	.955	.911	.451	.477	1.240	1.454	.441	.492	.223	.463	.178	.356	.233	.503	.210	.396
15	1.220	1.150	.508	.510	1.570	1.856	.473	.517	.362	.545	.260	.396	.308	.610	.257	.445
17	1.560	1.434	.532	.541	1.814	2.123	.481	.539	.536	.684	.298	.455	.491	.756	.338	.499
21	2.032	2.003	-	-	2.079	2.516	-	-	.725	.875	-	-	.644	.968	-	-
26	2.460	2.241	-	-	2.404	2.667	-	-	.960	1.115	-	-	.923	1.320	-	-
33	2.728	2.492	-	-	2.669	2.679	-	-	1.440	1.488	-	-	1.547	1.803	-	-

Growth in dry weight (g/ℓ) of Selenastrum capricornutum

TABLE 8. ANALYSIS OF 2^4 FACTORIAL EXPERIMENT
BASED UPON MAXIMUM GROWTH RATE PER UNIT VOLUME PER DAY

(g/ℓ-day)

	CO_2 Enriched Air								Non-CO_2 Enriched Air							
	350 ft-c				500 ft-c				350 ft-c				500 ft-c			
	600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium	
Rep.	Vent.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.
1	.230	.255	.084	.096	.282	.286	.108	.116	.103	.095	.068	.081	.095	.156	.024	.058
2	.190	.164	.072	.074	.188	.201	.088	.117	.087	.070	.041	.059	.092	.090	.041	.066

ANALYSIS OF VARIANCE

Main effects	F Ratio
CO_2 enrichment of air	89.72**
Light Intensity	2.92
Medium Concentration	98.88**
Method of air addition	1.86
Replicates	12.62**

Levels of significance
F(5%) = 4.54 *Significant at 5% level
F(1%) = 8.68 **Significant at 1% level

Interactions	F Ratio
CO_2 - Light Int.	2.11
CO_2 - Medium	24.40**
CO_2 - Air add.	0.17
Light Int. - Medium	1.20
Light Int. - Air add.	1.20
Medium - Air add.	0.42
CO_2 - Light-Medium	0.77
CO_2 - Light-Air add.	0.26
CO_2 - Medium-Air add.	0.02
Light-Medium-Air add.	0.13
CO_2 - Light-Medium-Air add.	0.19

TABLE 9. ANALYSIS OF 2^4 FACTORIAL EXPERIMENT
BASED ON MAXIMUM GROWTH RATE PER UNIT MASS PER DAY
(g/g-day)

	CO_2 Enriched Air								Non-CO_2 Enriched Air							
	350 ft-c				500 ft-c				350 ft-c				500 ft-c			
	600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium		600% Medium		100% Medium	
Rep	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.	Vert.	Aer.
1	.647	.658	.707	.586	.586	.578	.510	.648	.494	.623	.603	.728	.318	.399	.452	.440
2	.595	.613	.535	.670	.544	.609	.561	.613	.484	.588	.310	.528	.229	.365	.245	.429

ANALYSIS OF VARIANCE

Main effects	F Ratio
CO_2 enrichment of air	35.10**
Light Intensity	20.28**
Medium Concentration	0.33
Method of air addition	9.40**
Replicates	6.70*

Levels of significance
F(5%) = 4.54 *Significant at 5% level
F(1%) = 8.68 **Significant at 1% level

Interactions	F Ratio
CO_2 - Light Int.	7.48*
CO_2 - Medium	0.33
CO_2 - Air add.	2.72
Light Int. - Medium	0.56
Light Int. - Air add.	0.00
Medium - Air add.	0.20
CO_2 - Light-Medium	0.36
CO_2 - Light-Air add.	0.91
CO_2 - Medium-Air add.	0.02
Light-Medium-Air add.	0.00
CO_2 - Light-Medium-Air add.	0.55

TABLE 10. EVALUATION OF THE SIGNIFICANT FACTORS AFFECTING MAXIMUM NORMALIZED ALGAL GROWTH RATES

2^4 Factorial Experiment

(g/g-day)

Light Intensity	CO_2 Enriched Air	Non CO_2 Enriched	Total Effects (Avg. of CO_2 and Non CO_2 - Air)
350 ft-c	.626	.544	.585
500 ft-c	.581	.360	.471
Total Effects (Avg. of 350+500 ft-c)	.604	.452	

Air Addition	CO_2 Enriched Air	Non CO_2 Enriched	Total Effects (Avg. of CO_2 and Non CO_2 - Air)
Ventilation	.586	.392	.489
Aeration	.622	.513	.568
Total Effects (Avg. of vent. and aer.)	.604	.452	

Each value is the average of eight measurements (two replicates, four flasks per replicate) except for the total effects which are averages or sixteen measurements.

TABLE 11. EVALUATION OF THE SIGNIFICANT FACTORS AFFECTING VOLUMETRIC ALGAL GROWTH RATES

2^4 Factorial Experiment

(g/ℓ-day)

Medium Concentration	CO_2 Enriched Air	Non-CO_2 Enriched Air	Total Effects (Avg. of CO_2 and Non-CO_2 Enriched Air)
100%	.094	.054	.074
600%	.225	.099	.162
Total Effects (Avg. of 100% + 600% medium)	.160	.077	

Each value is the average of eight measurements (two replicates, four flasks per replicate) except for the total effects which are the average of sixteen measurements.

TABLE 12. EVALUATION OF THE SIGNIFICANT FACTORS AFFECTING ALGAL YIELDS

2^4 Factorial Experiment

(g/ℓ)

Days	CO_2 Enriched Air	Non-CO_2 Enriched Air
15	.514	.350

Each value is the average of eight measurements (two replicates, four flasks per replicate). Values for cultures grown in 100% medium concentrations only.

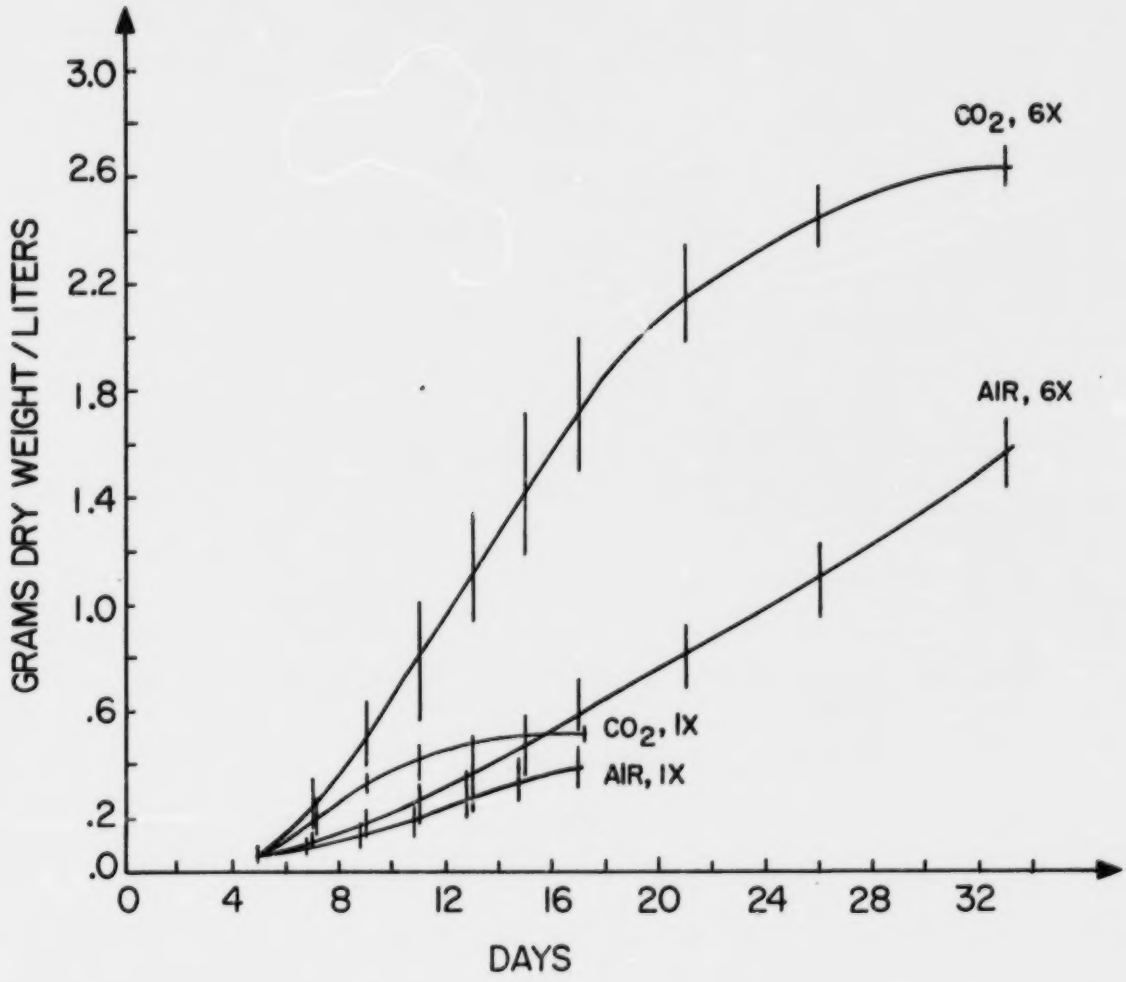

FIGURE 4. COMPOSITE RESULTS OF THE SIGNIFICANT FACTORS AFFECTING ALGAL GROWTH IN BATCH TESTS

Values expressed as dry weight (g/ℓ). Data from replicate two used for figure.

TABLE 13. EFFECTS OF CO_2 ENRICHMENT ON NUTRIENT CONCENTRATION

Element	CO_2 enriched	Non-CO_2 enriched
Iron	0.34	0.05
Manganese	3.35	2.58
Calcium	45.75	19.25
Silicon	1.68	8.85
Boron	0.55	1.13
Sodium	168.00	187.50

Analyses were made on 600% concentrated medium cultures of Selenastrum on the 33rd day after inoculation. Each value is the mean for four cultures. All values in ppm.

GLASSWARE PREPARATION

Glassware and all other culture vessel components were acid washed during the initial research period. In later experiments it was found that better replication was obtained if the wash water solution was modified to include 1-2% of hydrofluoric acid. This modification was included in all tests of the treatment plant effluents reported in this report.

STERILIZATION TECHNIQUES

All media for batch bioassays, either natural water, treated effluents or laboratory synthesized media must be pretreated in some way to remove naturally occuring organisms and ensure sterility. Several methods for accomplishing this were considered and evaluated by laboratory experimentation. These included autoclaving, ultra-violet irradation, exposure to ethylene oxide, tyndallization and filtration.

Autoclaving

Autoclave sterilization at 121°C and 15 pounds pressure for 15-20 minutes appears to be the method most suitable for most natural waters. Water samples sterilized by autoclaving should be placed in a pre-heated autoclave and removed as soon as the pressure is down in order to minimize heat damage to organic materials which may be present.

Ultraviolet Sterilization

The ultraviolet source used was enclosed in a wOoden box and a decastaltic pump was used to vary the flow rate of an algal suspension (3×10^5 cell/ml) past the ultraviolet coils. A length of two meters of coiled tubing was in close proximity to the ultraviolet source enclosed in the box. The lowest flow rate was 8.25 ml per hr. The results indicated that this method was not suitable for killing algae in a suspension of this density and further investigation of this method was terminated.

Ethylene Oxide

Autoclaving is difficult to apply in certain cases. Complex apparatus can be damaged by temperature changes such as occur during autoclaving or so large as to be unsuitable for sterilization by this procedure. Other methods have therefore to be applied for the elimination of contaminant organisms from apparatus prior to use. Ethylene Oxide has been used extensively for this purpose in medical bacteriology. Being gaseous permits adequate penetration of complex apparatus to be achieved. There are many problems associated with the use of Ethylene Oxide. The sterilization potential is reduced by the presence of water vapor in the apparatus and by polymerization. Unless removed the resulting powders and oily fluids are highly toxic to organisms introduced subsequently. Despit such drawbacks, the use of Ethylene Oxide as a means of sterilization is increasing steadily. Another serious problem is a consequence of its high toxicity to humans and the need for great care in handling. Even in a modern chemical laboratory, the fume hoods available were unsuitable for work with material of such toxicity and further consideration on this material for the sterilization of algal culture glassware had to abandoned.

Tyndallization

The problems of autoclaving are largely a consequence of the temperatures which have to be used. One way of partially circumventing these difficulties is to use the procedure referred to as "Tyndallization." The medium or apparatus is placed in water which is brought slowly to the boil and retained in a boiling condition for a period of 30 minutes. This slow heating to 100° C and retention at that temperature for 30 minutes is repeated for three successive days. Since this procedure is more time consuming and in some cases, maximum algal growth was not attained, it was not considered the best method for sterilizing natural waters.

Filtration

Filter sterilization would appear to be the ideal method for the sterilization of laboratory media in which all nutrients are in solution. Natural waters often contain large amounts of particulate matter which would be removed by the filtration process. Removal of particulate matter might be considered desirable or even necessary in laboratories utilizing electronic particle counters to monitor growth. Two types of filters were used in preliminary tests to determine to what extent the removal of the particulate material affected the algal growth potential of the water. The water used in this test was a secondary effluent from the Santee Treatment Plant. The two types of filters evaluated were Whatman #1 and Millipore 0.45µm. Four replicates were made of effluent filtered through Whatman #1, Millipore 0.45µm and growth compared with four

replicates of nonfiltered effluent which were autoclave sterilized. Culture vessels were 500 ml Erlenmeyer flasks filled to 250 ml with effluent. Flasks which were to contain the filtered effluent were sterilized and the effluent aseptically transferred from the filtration apparatus to these flasks. Initial cell concentration of Selenastrum capricornutum was 1000 cell/ml and Air - CO_2 mixture used to control pH. The results of this experiment are shown in Table 14.

EVALUATION OF MATERIALS FOR ALGAL CULTURE SYSTEMS

Many different materials have been proposed and/or used to construct systems for both batch and continuous algal cultures. During the early part of this research it became clear that some materials could inhibit algal growth in these systems and thus give biased results in evaluation of effluents. An example of the effect of one such material is shown in Figure 5. Based on these limited observations it was decided that all types of material intended for the test units should be evaluated prior to use.

A modified 2^4 factorial experiment was designed to test the effects of rubber stoppers (Neoprene vs. Cafe-au-lait (Rhoades Rubber Mfg. Co.)).

TABLE 14. COMPARISON OF THE EFFECT OF AUTOCLAVING AND OF FILTRATION ON THE MEASURED GROWTH POTENTIAL OF SANTEE SECONDARY EFFLUENT

NUMBER OF CELLS PER Mℓ

Growth Day	Autoclaved Non-Filtered	Filtered Millipore (.45μ)	Filtered Whatman #1
17	3.43×10^6	6.81×10^5	9.55×10^5
23	2.05×10^6	7.48×10^5	7.79×10^5

TOTAL CELL VOLUME μm^3 per mℓ

Growth Day	Autoclaved Non-Filtered	Filtered Millipore (.45μ)	Filtered Whatman #1
17	1.94×10^8	4.00×10^7	5.27×10^7
23	1.03×10^8	4.03×10^7	4.10×10^7

Each datum an average of 4 flasks.

T-tests on this data indicate no significant difference between the two types of filtration but a highly significant (0.1% level) difference between filtered and nonfiltered effluent. The results indicate that filtration removed significant quantities of nutrients, and it was therefore decided not to use filtration as a means of sterilization when a maximum biomass measurement is desired.

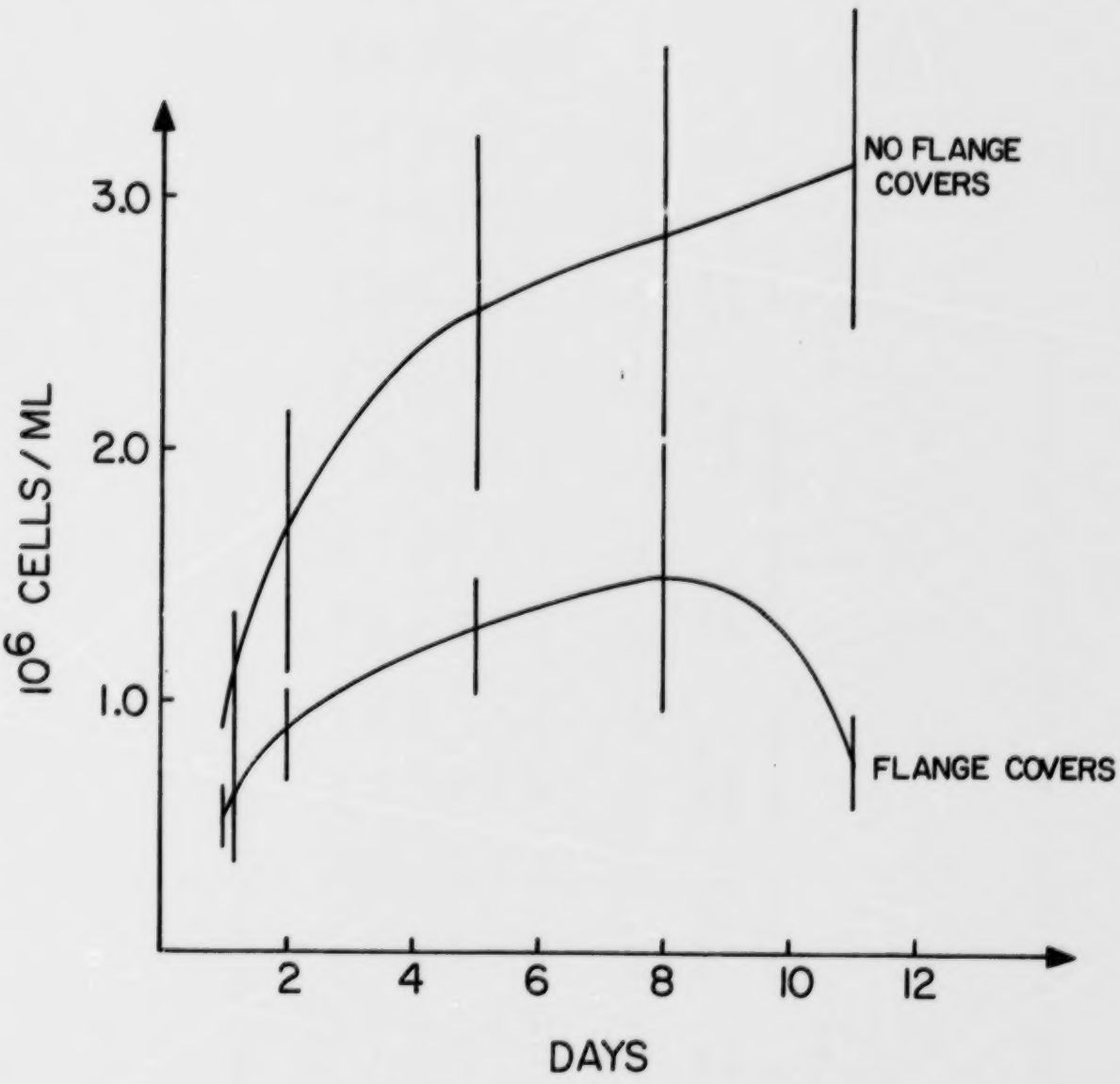

FIGURE 5. THE EFFECTS OF PLASTIC FLANGE COVERS ON ALGAL GROWTH IN TEST UNITS

TABLE 14. EFFECT OF NONSOAKED MATERIALS ON ALGAL GROWTH

	PYREX								KIMAX							
	NEOPRENE STOPPERS				CAFE-AU-LAIT STOPPERS				NEOPRENE STOPPERS				CAFE-AU-LAIT STOPPERS			
	Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing	
Day	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
5	.052	.053	.042	.042	.026	.037	.021	.029	.063	.051	.056	.045	.027	.022	.026	.021
8	.222	.228	.240	.240	.027	.026	.023	.014	.228	.262	.240	.216	.040	.026	.081	.068
10	.226	.215	.294	.294	.005	.023	.002	.010	.228	.270	.335	.264	.025	.042	.155	.181
12	.333	.290	.366	.366	.042	.053	.040	.055	.281	.399	.373	.369	.090	.169	.294	.306
15	.416	.376	.461	.461	.088	.080	.168	.178	.391	.410	.480	.418	.269	.185	.427	.362
19	.401	.377	.482	.482	.050	.045	.200	.056	.405	.449	.510	.483	.257	.259	.414	.482

Growth in dry weight (g/ℓ) of Selenastrum capricornutum

TABLE 15

ACCURACY AND PRECISION OF ANALYTICAL METHODS
AT DIFFERENT CONCENTRATION LEVELS

COMPOUND	MEAN	COEFFICIENT OF VARIATION (%) AT EACH CONC. LEVEL a)
Fe	1	100
(µg/ℓ)	5	20
	31	11
Mn	2	30
(µg/ℓ)	49	31
	135	3
NO_2	.039	31
(mg/ℓ)	.260	3
	1.14	2
NO_3	.030	80
(mg/ℓ)	.390	24
	2.15	12
P	.112	21
(mg/ℓ)	.200	22
	3.52	9
	9.76	2

a) coefficient of variation = 100 (standard deviation)/(mean)

TABLE 16. EFFECT OF SOAKED MATERIALS ON ALGAL GROWTH

	PYREX								KIMAX							
	NEOPRENE STOPPERS				CAFE-AU-LAIT STOPPERS				NEOPRENE STOPPERS				CAFE-AU-LAIT STOPPERS			
	Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing		Surgical Tubing		Regular Tubing	
Day	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
5	.093	.141	.101	.107	.103	.103	.096	.096	.123	.129	.128	.119	.109	.130	.114	.111
8	.253	.287	.246	.290	.212	.212	.251	.187	.300	.215	.314	.242	.282	.261	.234	.249
10	.341	.357	.370	.421	.309	.309	.369	.244	.379	.343	.386	.363	.361	.327	.331	.374
17	.572	.577	.607	.605	.534	.534	.576	.401	.559	.571	.598	.562	.540	.524	.559	.515
23	.501	.501	.559	.537	.525	.525	.517	.369	.517	.417	.540	.536	.368	.440	.449	.385

Growth in dry weight (g/ℓ) of Selenastrum capricornutum

TABLE 17. FACTORIAL ANALYSIS OF COMBINED SOAKED AND NONSOAKED MATERIAL EXPERIMENTS

(g/ℓ)

	NONSOAKED MATERIALS								SOAKED MATERIALS							
	PYREX GLASSWARE				KIMAX GLASSWARE				PYREX GLASSWARE				KIMAX GLASSWARE			
	NEOPRENE STOPPERS		CAFE-AU-LAIT STOPPERS		NEOPRENE STOPPERS		CAFE-AU-LAIT STOPPERS		NEOPRENE STOPPERS		CAFE-AU-LAIT STOPPERS		NEOPRENE STOPPERS		CAFE-AU-LAIT STOPPERS	
Replicates	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing	Surg. Tubing	Reg. Tubing
Rep. 1	.226	.294	.005	.002	.228	.335	.025	.155	.341	.370	.309	.369	.379	.386	.361	.331
Rep. 2	.215	.294	.023	.010	.270	.264	.042	.181	.357	.421	.309	.299	.343	.363	.327	.374

ANALYSIS OF VARIANCE

Main Effects	F Ratio
Soaking Treatment	424.45**
Glassware	12.17**
Rubber Stoppers	173.59**
Plastic Tubing	21.34**
Replicates	0 02

Levels of Significance

F(5%) = 4.54* Significant at 5% level
F(1%) = 8.68** Significant at 1% level

Interactions	F Ratio
Soaking - Glassware	5.29*
Soaking - Stoppers	88.33**
Soaking - Tubing	4.42
Glassware - Stoppers	7.86*
Glassware - Tubing	0.88
Stoppers - Tubing	0.10
Soaking-Glassware-Stoppers	1.28
Soaking-Glassware-Tubing	5.10*
Soaking-Stoppers-Tubing	0.17
Glassware-Stoppers-Tubing	5.94*
Soaking-Glassware-Stoppers-Tubing	3.95

TABLE 18. EFFECT OF NONSOAKED MATERIALS BASED ON ALGAL YIELDS (g/ℓ) ON THE TENTH DAY

Data Analyzed as a 2^3 Factorial Experiment

ANALYSIS OF VARIANCE

MAIN EFFECTS	F RATIO	INTERACTIONS	F RATIO
Glassware	65.30**	Glassware-stoppers	30.21**
Rubber Stopper	993.17**	Glassware-tubing	19.88**
Plastic Tubing	88.07**	Rubber stoppers-tubing	0.02
Replicates	0.15	Glassware-stoppers-tubing	38.21**

Levels of Significance

F(5%) = 5.59* Significant at 5% level
F(1%) - 12.25** Significant at 1% level

TABLE 19. EFFECT OF SOAKED MATERIALS BASED ON ALGAL YIELDS (g/ℓ) ON THE TENTH DAY

Data Analyzed as a 2^3 Factorial Experiment

ANALYSIS OF VARIANCE

MAIN EFFECTS	F RATIO	INTERACTIONS	F RATIO
Glassware	1.81	Glassware-stoppers	3.58
Rubber Stoppers	18.31**	Glassware-tubing	2.24
Plastic Tubing	8.12*	Rubber stoppers-tubing	0.69
Replicates	0.32	Glassware-stoppers-tubing	0.26

Levels of Significance

F(5%) = 5.59* Significant at 5% level
F(1%) - 12.25** Significant at 1% level

plastic tubing (Tygon Surgical Grade (S-50-HL) vs Tygon Regular Grade (R-3603)), glass (Kimax vs Pyrex) and soaking treatment prior to use (pre-soaked material vs nonsoaked materials). Substances were added as thin section to either Kimax or Pyrex 1ℓ Erlenmeyer flasks. The results of these experiments are presented in Tables 15 and 16. The experiments were analyzed statistically on the basis of algal yields on the tenth day after inoculation. These analyses were run in two ways:

1. combined as a 2^4 factorial experiment
2. separately as two 2^3 factorial experiments.

The results of these analyses are presented in Table 17 and 18, 19 respectively. All of the nonsoaked materials were shown to exert significant effects on algal growth (Table 18). These effects were inhibitory with the effects of Pyrex > Kimax, Surgical tubing > Regular tubing, Cafe-au-lait > Neoprene. The soaking pretreatment significantly reduced the overall inhibitory effects (Table 17) and eliminated the difference between Pyrex and Kimax, but did not eliminate the differences between the two types of stoppers or the two types of tubing (Table 19). Interactions between the nonsoaked materials were shown to be present (Tables 17, 18) but these effects were eliminated by the soaking pretreatment (Table 19). Based on these results it was decided to use only Pyrex glassware and surgical grade Tygon tubing in the algal assay units.

FINAL BATCH TEST PROCEDURE

Following the completion of the testing and procedure development described above, the final batch procedure described in Reference IV-1 was adopted and used in the testing of all treatment plant effluents.

As a part of this research, the UCI Laboratory also participated in comprehensive inter-laboratory evaluations of the procedure under the guidance of the Pacific Northwest Water Laboratory, U.S. Environmental Protection Agency.

SECTION V

CONTINUOUS CULTURE ASSAY METHODS

CHEMOSTAT DESIGN

The continuous culture units proposed in the Provisional Algal Assay Procedures (Reference 2) were modified on the basis of the results obtained as described in Section IV. The most important modifications included the use of CO_2-air for pH control and the redesign of the units such that the only three materials in contact with the cultures were Pyrex glass, Surgical grade Tygon tubing and Teflon. Soaked Neoprene stoppers were used to top the culture units but were not in direct contact with the growing cultures.

The equipment needed for eight continuous culture tubes are listed in Table 20 and a typical set of four assembled units are shown on Figure 7.

CHEMOSTAT OPERATION

Before each new experiment was started, chemostats including feed and air tubes were autoclaved at 121°C for 15 min. The chemostats were then filled with sterilized media and inoculated to 10^3 cells of Selenastrum per ml. Flow was initiated after significant algal growth was observed.

Because the secondary and tertiary effluents sometimes contained considerable particulate matter, the feed pumps were set up to operate for a short time each hour at a high flow rate to prevent clogging of feed tubes. The pumps were calibrated to operate at 50 ml per hour per feed tube and the time was set to have the pump on 1/12, 1/8 or 1/4 of an hour each hour. This resulted in residence times of approximately 10, 6 and 3 days respectively. Throughout the experiments no problems were found with this method.

Three parameters of biomass were observed during each experiment: dry weight, cell volume and cell number. Samples needed for determination of cell volume and number were taken directly from the chemostats through sampling ports at the base of each unit. Effluents in the overflow flasks were used for dry weight measurements in order to minimize disturbance of the systems.

A continuous record of the residence times was kept during the experiment. This was done by weighing the feed flasks and overflow flasks everytime either a dry weight sample was taken, or more feed medium was added. Residence times at steady state were computed from the flow during steady state and the time of steady state. Steady state was defined to have been reached when chemostats maintained a constant cell mass for periods of time greater than one residence time with only random fluctuations. Near the end of the steady state period samples from the overflow flasks were collected for chemical analysis.

From preliminary tests with chemostats it was found there was a problem of algae sticking to the culture vessel, especially at the air/water interface. A strong magnet was used to guide the magnetic spin bar inside the tubing to scrap the inside walls. With this procedure it was never necessary to remove the plug at the top of the chemostat.

After some length of operation feed tubes and overflow tubes may become partly clogged with particulates, bacteria, and algae. The feed tubes were changed twice during the 195 days of operation and the overflow tubes were changed once. New feed tubes were autoclaved before being put into use. Feed tubes from the pump to the chemostat have a tendency to get algae growing in them, even though the medium is released 10 cm above the water level.

The overflow flasks may become coated with algae if overflow effluent is allowed to remain too long in the flasks.

TABLE 20

EQUIPMENT FOR EIGHT CONTINUOUS CULTURE SYSTEMS

2 (or 1) pumps:	Buchler Dekastaltic Pump, Model No. 2-6500
8 Culture Tubes:	Pyrex glass 5 cm i.d. 60 cm length with glass tube near bottom and another for overflow at a volume of 950 ml or 50 cm from base.
Surgical Grade Tygon^{R} Tubing	Feed tubes 1/16 in (.16 cm) i.d., 1/8 in (.32 cm) o.d. Air lines 3/16 in (.48 cm) i.d., 5/16 in (.79 cm) o.d.
Pump Tubes:	Auto Analyzer^{R} Pumps .065 in (.165 cm) i.d., Technicon Corporation
Stoppers:	Neoprene Size #11 for top of culture tube Size #9 for top of overflow flasks
Nalgene:	Tube connectors and stopcocks used as valves
8 Fluorescent lamps:	30 watt "cool-white" G.E.
12 Magnetic Mixers:	Thermolyne
2 Air Pumps:	Silent Giant
30 1000 ml Erylmeyer Flasks	Pyrex, for feed storage, feed flasks, and overflow flasks
1 Cam timer:	Minarik Recycling Timer Model 5C BR-B-60 (one revolution per hour)

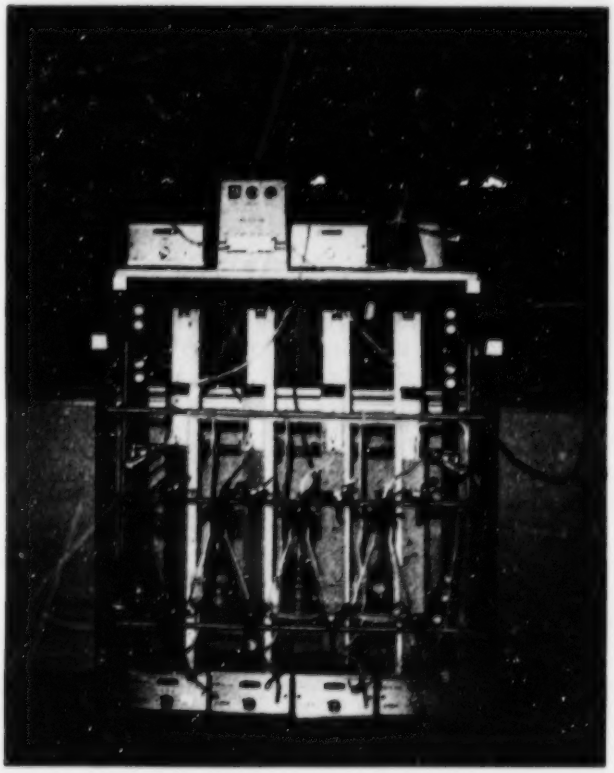

FIGURE 6 - CONTINUOUS CULTURE UNIT

SECTION VI

FIELD TEST SYSTEMS

Application of the chemostat, continuous culture concept to ponds of much greater volume than a laboratory chemostat tube provide a transition to those natural situations where effluents of different types are discharged into natural bodies of water. In this way natural populations of many algal species may be evaluated under realistic field conditions. The algal flora in a pond receiving a specifically treated, and presumably well defined, effluent might be expected to display uniform behavior, particularly in an area such as Santee, California where atmospheric variables are as stable and predictable as any to be found. Furthermore, the flora of ponds receiving effluents from different treatments might be expected to show certain specific differences.

Two study ponds were established in the location between the treatment plant and the lakes. The operating parameters for the two ponds were as follows:

POND NO.	SOURCE	VOLUME (gal.)	DEPTH (ft.)	FLOW (gal/min)	RESIDENCE TIME (days)
1	Secondary Effluent	116,000	2.5	10	8.02
2	Tertiary Effluent	124,000	2.6	10	8.62

A typical study pond is shown in Figure 8. Preliminary samples were obtained from the study ponds during the spring and early summer months (1971) in order to become familiar with algal floras. Samples were collected, generally in the late morning, from the midpoint on the shore on each side of the pond by means of a plastic container on a long rod. These four sub-samples were then combined and examined microscopically in order to determine the organisms present.

The organisms survey were limited to those suspended in the water because filamentous material did not develop except on a few stones near the inflow pipe. This was fortunate, in that assessment of the biomass of epiphytic and epilithic periphyton is notoriously difficult to undertake in any meaningful manner.

Examination during the preliminary phase of monitoring the pond flora was done with a compound microscope. Quantification was estimated by scanning slides and judging whether the organism was "rare" - "occasional" - "frequent" or "abundant." During the latter part of the investigation, the number of organisms present were quantified more precisely by using a Nikon inverted microscope, Model MS. A 5-ml aliquot of the undiluted pond sample was pipetted into a Zeiss counting chamber and a second replicate made. The organisms were settled by adding drops of iodine. Counting was accomplished

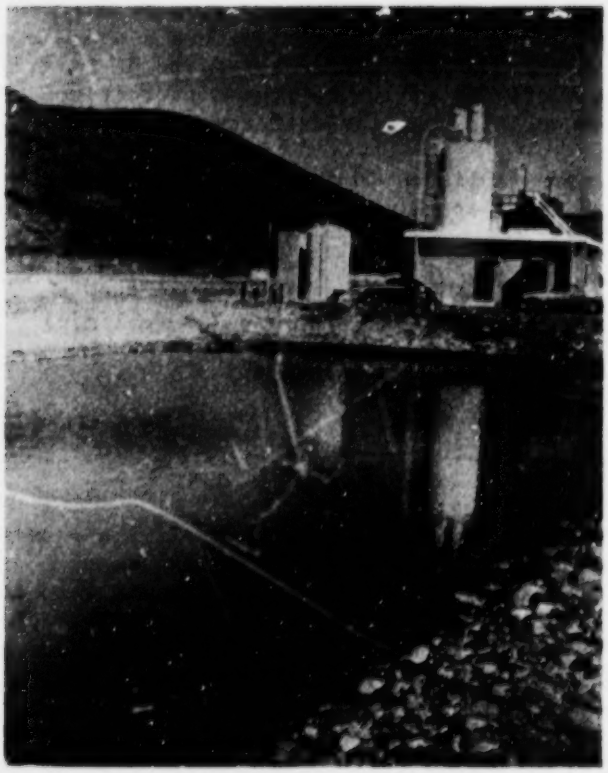

FIGURE 7 - TYPICAL STUDY POND

with Wild oculars containing a millimeter grid rules 10 squares to a side. Ten strips of 10 squares were counted at random for the 5-ml aliquot and the organisms recorded. The results were expressed in numbers of organisms per liter. Determinations of total biomass based on dry weight measurements were conducted in accordance with Standard Methods. Estimates of color were made visually.

For measurement of turbidity a procedure equivalent to that of Secchi-disc determination was used although the disc employed was not standard. This was placed in the ponds and the depths at which it disappeared noted. The measurements were made at mid-day, in sunlight, at the middle of the south sides of the secondary and tertiary ponds.

SECTION VII

EVALUATION OF TREATMENT PROCESSES

The original investigation plan called for evaluation of the tertiary demonstration process for phosphate removal in a modified activated sludge process at the Irvine Ranch Water District.

During the first phase of this investigation when the testing procedures were being developed it became clear that the objective could not be reached because of delays in the Irvine Ranch Water District's demonstration project. Consequently, it was decided instead to evaluate the different tertiary effluents from the tertiary demonstration project at Santee County Water District. In addition to the time advantage, this change also was advantageous becuase it permitted the expansion of the evaluation program to include effluents for an electrodialysis process and effluents from a ground percolation process. In order to accomplish this modified plan of investigation, an additional $9,000 was obtained from the Santee County Water District's demonstration grant. At the same time it was also decided that the data obtained and presented below would also be made part of the report of the District's demonstration grant report (Reference VII-1).

GENERAL OBJECTIVES

The general objectives of the treatment process evaluation were:

1. To determine the relative and absolute effectiveness of the tertiary treatment processes with respect to removal of algal nutrients, and
2. To determine the probable response of artificial lakes and water reservoirs to the tertiary effluents from these treatment processes.

SPECIFIC OBJECTIVES

The specific objectives of this investigation were:

1. To determine the growth limiting inorganic nutrient(s) in:
    a. Secondary (act. sludge) Effluent.
    b. Tertiary (lime treated) Effluent.
    c. Electrodialysis Effluent.
    d. Secondary Effluent after Ground Percolation.
2. To determine the sustained level of algal growth under standard laboratory conditions in continuous culture for:
    a. Secondary (act. sludge) Effluent.
    b. Tertiary (lime treated) Effluent.
    c. Electrodialysis Effluent.
3. To determine the level of algal growth in study ponds for the secondary and tertiary effluents.
4. To compare the laboratory and study pond results and determine if laboratory tests can be used to predict the response of an artificial lake receiving a given effluent.

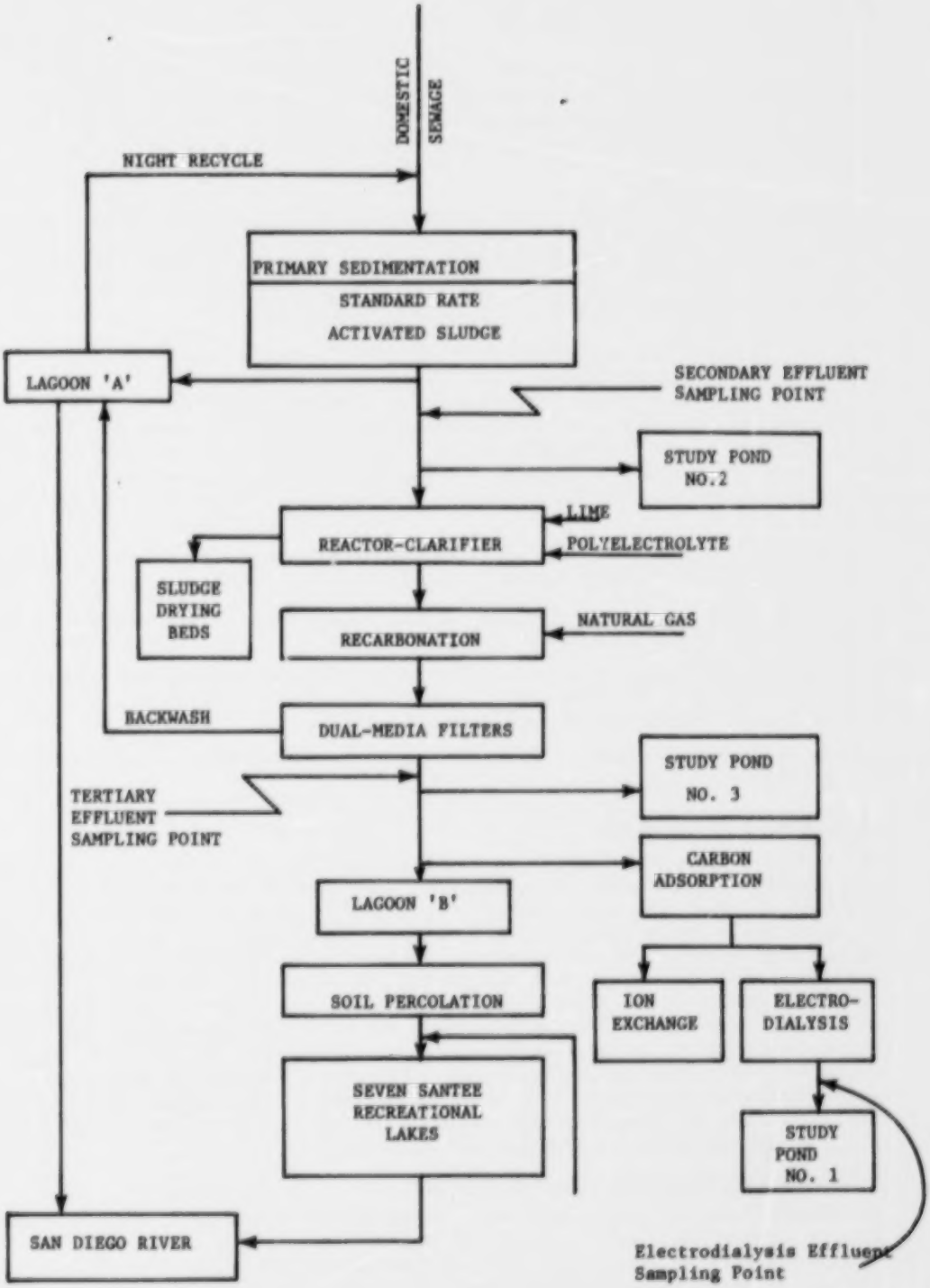

FIGURE 8 - ALGAE GROWTH STUDY SAMPLING POINTS

METHODS AND PROCEDURES

Sampling

Samples were collected at the Santee Treatment Plant in February, June and August 1971. The sample points are shown in Figure 9. The sample collection corresponded to specific modes of plant operation as indicated in the schedule shown below.

Secondary Effluent (1) Grab sample from inlet pipe to tertiary lime reactor-clarifier on 26 February 171. A sample representative of normal secondary effluent. (2) 24 hour composite sample from inlet pipe to tertiary lime reactor-clarifier, on 21-22 June 1971. A sample representative of secondary effluent and typical influent to the tertiary plant.

Tertiary Effluent (1) Grab sample of tertiary discharge after phosphate removal, pH adjustment and sand filtration on 26 February 1971. (2) Composite sample over 24 hour period from the lime reactor-clarifier. No pH adjustment has been made at this point in the treatment process and pH of this effluent was 9.8. (3) Grab sample after pH adjustment and filtration The sand filters were back-washed for 1/2 hour before the sample was collected. This sample representative of typical effluent from the tertiary plant.

Electrodialysis Effluent (1) Composite sample collected after lime treatment recarbonation, filtration, carbon adsorption and electrodialysis.

Ground Percolated Effluent (1) Grab sample after tertiary treatment, lagoon detention and normal percolation of 400' in sand and gravel (on 21 June).

Samples were collected at Santee in Pyrex bottles, autoclaved and stored in the dark at 4 ± 1°C until used. The four 5 gallom samples of each effluent were thoroughly mixed before autoclaving.

Sample Pretreatment Samples were pretreated by autoclaving as discussed in Section IV.

Continuous Culture Assays

The composite sample collected on 21-22 June were used throughout the entire experiment. For purposes of comparison, the standard batch reference medium (Section IV) was also used. The Santee samples were stored and used as required while the reference medium was made up in batches from time to time during the course of the experiment. The test alga, Selenastrum capricornutum was inoculated into chemostats containing one of the Santee effluents or the reference medium at an initial concentration of 10^3 cells per ml. Duplicate chemostats were set up for each effluent and the reference medium. The chemostats were run until a steady state was achieved. (Steady state was defined as less than 10% variation in cell concentration per week.) The chemostats were maintained at a constant residence time of 10.5 days. This residence time was approximately equal to the hydraulic residence time in the study ponds described below under the field tests.

Determination of Growth Limiting Nutrients

The growth limiting nutrient(s) were determined by means of factorial spiking experiments as described by Murray et al 1971 (VII-2) and in Reference IV-1). "Factor" refers to the element (or combinations of elements added as a single factor) as shown for each experiment. Two sets of spiking experiments were conducted on each type of effluent.

The first spiking experiments were designed to determine the group to which the growth limiting nutrient belonged. The nutrients investigation were classified in the following four groups:

1. Micronutrients: B, Zn, Co, Cu, Mo
2. Iron and Manganese
3. Phosphorus and Nitrogen
4. Macronutrients: Ca, Mg, Na, K, Cl, S, C

Data from the first spiking experiments were used to determine four individual elements which appeared most significant and a second set of factorial experiments were then designed to evaluate these four individual growth limiting nutrients. The four elements selected for the second set of experiments were:

1. Phosphorus
2. Iron
3. Nitrogen
4. Manganese

Spiking Concentrations

Spikings were performed by addition of specified amounts of concentrated stock solutions of macro- and micronutrients such that the final spiking after dilution into the effluent being tested corresponded to 30 percent of the concentration of the reference medium described above. For example, spiking with iron was done such that 11 µg Fe was added per liter of effluent being tested.

Evaluation Parameters

Three different biomass parameters were used to determine algal growth: cell count, total cell volume and dry weight.

Biomass can be expressed in various ways. Measurement of dry weight is useful in terms of energy values, cell numbers are of significance from a turbidity and analytical point of view, and total cell volume is a useful parameters which may combine the advantages of cell counts and dry weight. Measurements of cell volume correlate best with optical density, what the eye actually sees, and this may be the most useful assessment of algal growth, particularly by the nontechnical viewer.

CHEMICAL ANALYSES

The 21-22 June composite samples and reference medium were analyzed for iron, manganese, nitrite, nitrate and phosphate.

Methods for analysis of nitrites, nitrates, and total phosphorus are those given in FWPCA Methods for Chemical Analysis of Water and Wastes, 1969 (VII-3).

GROWTH LIMITING NUTRIENTS

Determination of the growth limiting nutrients was a key aspects of the evaluation of the various treatment process effluents. This determination was considered of special significance in this investigation because the various treatment processes do remove different nutrients in unequal

proportions. When considering process improvements, it is therefore necessary to know which specific nutrient (or nutrients) are limiting growth of algae. The batch assay evaluation in this investigation for the Santee County Water District included only the limiting nutrients as they affect the maximum algal biomass. This choice of approach was based on the specific situation at Santee where all wastes are discharged in lakes having hydraulic residence times in excess of ten days. Basing the determination of growth limiting nutrients on continuous culture experiments was considered. However, because limiting nutrients change as a function of residence/growth time and the need to evaluate and screen many potential limiting nutrients and their interactions, it appeared that factorial multinutrient batch testing would provide a more comprehensive evaluation.

Reference Medium - The results of the determination of groups of growth limiting nutrients are summarized in Table 21. The corresponding summary results of the evaluation of single nutrients are shown in Table 22.

The groups spiking experiment (Table 21) showed that addition of (Fe + Mn) as a group resulted in an increase in the number of algal cells. The increase amount of $1.12\ 10^6$ cells per ml or of 23 percent and the increase was statistically significant at the 95 percent level. However, the addition of (Fe + Mn) did not result in a statistically significant increase in either cell volume or dry weight despite the increase in cell numbers.

Both (P + N) and macronutrients limit growth in the reference medium as measured by all three parameters used, cell number, cell volume, and dry weight. Addition of (P + N) shows a greater effect on the final growth than does the addition of macronutrients.

The individual nutrient spiking experiment was based on the results of the group spiking experiment and four individual nutrients were tested. The four nutrients were Fe, Mn, P, N. The results in Table 22 show that of those four nutrients only phosphorus appears to limit growth in the unspiked medium when all three biomass indicators are considered. Spiking with iron results in a slight decrease in final biomass as measured by both cell number and cell volume. This suggests that iron might inhibit growth in the reference medium; however, more work would be necessary prior to determining whether this is a supportable conclusion.

Secondary Effluent

Secondary effluent samples collected on two different dates were evaluated in the group spiking experiment. The chemical composition of these effluents are shown in Table 23. The results for the first sample (collected on 26 February 1971) are shown in Table 25. The only nutrient group to have a significant effect was (Fe + Mn). The (Fe + Mn) addition resulted in an increased biomass for all three parameters, thus indicating that the growth of algae in that effluent sample was limited by either iron or manganese.

TABLE 21

REFERENCE MEDIUM TESTING OF NUTRIENT GROUPS

EFFECT OF SPIKING

Nutrient Group Spiked	Cell Number (No/mℓ) With (a)	Cell Number (No/mℓ) Without (b)	Total Cell Volume ($mm^3/ℓ$) With (a)	Total Cell Volume ($mm^3/ℓ$) Without (b)	Dry Weight (mg/ℓ) With (a)	Dry Weight (mg/ℓ) Without (b)
Trace Metals	5.67×10^6	5.16×10^6	323	293	197	177
Fe + Mn	5.97×10^6	4.85×10^6	322	294	190	184
P + N	6.57×10^6	5.07×10^6	369	247	223	151
Macro-nutrients	5.55×10^6	5.28×10^6	332	284	201	172

SIGNIFICANT FACTORS ON FINAL DAY

Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)		* (c)		+
(Fe + Mn)	**			+
(P + N)	**	**	**	+
(MACRO)	*	**	*	+

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 22

REFERENCE MEDIUM TESTING OF INDIVIDUAL NUTRIENTS

Added Single Nutrient	EFFECT OF SPIKING					
	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
P	5.33×10^6	3.74×10^6	299	255	136	121
Fe	4.39×10^6	4.67×10^6	265	289	127	130
N	4.50×10^6	4.56×10^6	283	270	130	127
Mn	4.65×10^6	4.42×10^6	287	266	127	130

SIGNIFICANT FACTORS ON FINAL DAY

FACTOR	CELL NUMBER	CELL VOLUME	DRY WEIGHT	COMPARISON
P	**	**	**	+
Fe	*	*		-
N				
Mn		**		+

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 23

CHEMICAL COMPOSITION OF SECONDARY EFFLUENTS

Constituent	DATE OF SAMPLING 26 Feb 71	21 June 71
Phosphorus mg P/ℓ	11.8	8.85
Nitrate mg N/ℓ	0.9	4.4
Nitrite mg N/ℓ	3.6	0.37
Kjeldahl Nitrogen mg N/ℓ	8.4	14.7
N/P ratio	1.09	2.20
Iron µg/ℓ	No data	1 µg/ℓ ± 1
Manganese µg/ℓ	No data	7 g/ℓ ± 1

The results for the 21 June 1971 sample are shown in Table 26, also showed an increased biomass as measured by cell number and total cell volume (but not dry weight) with the addition of the (Fe + Mn) groups. However, for this sample of secondary effluent the (P + N) spike resulted in a significant increase for all three parameters of growth. These results indicate that the secondary sample from 21 June has received a different degree of treatment than the sample collected 26 February 1971.

TABLE 24

CHEMICAL COMPOSITION OF TERTIARY EFFLUENTS

Constituent	DATE OF SAMPLING 26 Feb 1971	21 June 1971	25 Aug 1971
Phosphorus mgP/ℓ	0.77	.280	.21
Nitrate mg N/ℓ	1.10	4.3	
Nitrite mg N/ℓ	0.16	< 1.0	< 1.0
Kjeldahl Nitrogen mg N/ℓ	4.6	7.82	
N/P ratio	7.61	46.86	
Iron µg/ℓ	No data	< 1	< 1
Manganese µg/ℓ	No data	1	1

The individual nutrient spiking was made only on the 21 June 1971 sample and results as shown in Table 27 indicate that nitrogen is the limiting nutrient for all three growth parameters. Spiking with the nutrients P and Mn result in decreased biomass for all parameters. The Fe addition has a slight but insignificant increase for cell number and volume on the final day. However, analyses of the detailed results indicated that Fe addition results in increased cell number and volume early in the experiment, i.e. affecting growth rate but not final biomass. The number of samples and the single source of effluent limit the conclusions which can be drawn, it is therefore recommended to expand the investigations to

include other secondary effluents and more samples of each effluent.

Tertiary Effluent

Three samples of tertiary effluent were collected under different treatment plant operating conditions and the growth limiting nutrients determined. The chemical compositions of these effluents are shown in Table 24.

Results of the 26 February sample (Table 28) show that the (P + N) addition results in an increased total cell volume but that the other parameters are not affected significantly. At the same time, the (Fe + Mn) addition results in a decrease in cell numbers. It should be noted that both effects, although statistically significant, are numerically small.

The 21 June sample was taken directly from the lime reactor-clarifier tank because of operating difficulties with the filters in the treatment plant. The sample contained large amounts of flocculated material which appeared to inhibit growth of the test organism. Data from the chemostats described later in the report indicate that batch experiments did not go to completion and that maximum biomass was not attained even after 58 days. Also there was a significant difference between the replicate flasks for the dry weight parameter. Consequently the results have not been considered in detail.

The results of the group and individual nutrient spiking experiments for the 25 August sample are presented in Table 29 and 30. Group spiking showed both (Fe + Mn) and (P + N) limiting for all three growth parameters. Spiking with the single nutrients showed that P and Fe were both limiting while N and Mn additions inhibited growth significantly.

Electrodialysis Effluent

The chemical composition of the electrodialysis effluent is given in Table 31. Results of the nutrient group spiking experiment are shown in Table 32. The addition of the (Fe + Mn) group resulted in a very significant increase for all three growth parameters while the addition of the macroelement group resulted in a decrease in the final total cell volume and dry weight. This again indicates the potential importance of (Fe + Mn) as growth limiting nutrients.

Results of spiking with the single nutrients are shown in Table 33. Although addition of both Fe and P result in highly significant increases for all biomass parameters, the significance for iron is much greater. The N spike resulted in a significant decrease for all biomass parameters while the Mn caused a decrease in dry weight only. Examinations of the detailed factorial analysis indicated that the numerical effect of the P and N additions are about the same, one increasing growth and the other decreasing growth. Therefore, spiking with the combination of these two nutrients as in the group testing experiments caused no significant effect. This indicates the caution required in interpreting results both from spiking with groups of nutrients and from evaluation of gross effects of treatment process modifications. In both cases conclusions should be based on overall results plus assessment of growth limiting nutrients.

TABLE 25

TESTING OF NUTRIENT GROUPS - SECONDARY EFFLUENT - 26 Feb

EFFECT OF SPIKING

Nutrient Group Spiked	Cell Number (No/mℓ) With (a)	Cell Number (No/mℓ) Without (b)	Total Cell Volume ($mm^3/ℓ$) With (a)	Total Cell Volume ($mm^3/ℓ$) Without (b)	Dry Weight (mg/ℓ) With (a)	Dry Weight (mg/ℓ) Without (b)
Trace Metals	3.35×10^7	3.99×10^7	1650	2186	874	909
Fe + Mn	5.43×10^7	1.92×10^7	2518	1618	1091	692
P + N	3.98×10^7	3.38×10^7	2005	2041	904	879
Macro-nutrients	4.08×10^7	3.27×10^7	2141	1905	932	850

SIGNIFICANT FACTORS ON FINAL DAY

Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)				
(Fe + Mn)	**	**	**	+
(P + N)				
(MACRO)				

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 26

TESTING OF NUTRIENT GROUPS - SECONDARY EFFLUENT 21-22 June

	EFFECT OF SPIKING					
	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
Nutrient Group Spiked	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
Trace Metals	$2.[illegible] \times 10^7$	2.16×10^7	1326	1246	4[illegible]4	441
Fe + Mn	$2.[illegible] \times 10^7$	$2.1[illegible] \times 10^7$	1[illegible]47	1224	474	433
P + N	2.33×10^7	2.10×10^7	13[illegible]8	1203	4[illegible]7	440
Macro-nutrients	$2.0[illegible] \times 10^7$	2.37×10^7	123[illegible]	1332	435	472

SIGNIFICANT FACTORS ON FINAL DAY				
Factor.	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)				
(Fe + Mn)	*	**		+
(P + N)	*	**	*	+
(MACRO)	**	*		

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 27

TESTING OF INDIVIDUAL NUTRIENTS - SECONDARY EFFLUENT 21-22 June

	EFFECT OF SPIKING					
Added Single Nutrient	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
P	1.43×10^7	1.59×10^7	820	913	415	457
Fe	1.53×10^7	1.49×10^7	867	866	432	440
N	1.56×10^7	1.46×10^7	908	825	451	421
Mn	1.37×10^7	1.65×10^7	775	958	377	495

SIGNIFICANT FACTORS ON FINAL DAY

FACTOR	CELL NUMBER	CELL VOLUME	DRY WEIGHT	COMPARISON
P	**	**	**	
Fe				
N	*	**	*	+
Mn	**	**	**	-

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 28

TESTING OF NUTRIENT GROUPS - TERTIARY EFFLUENT 26 Feb

	EFFECT OF SPIKING					
	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
Nutrient Group Spiked	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
Trace Metals	4.37×10^7	4.31×10^7	1975	1956	888	914
Fe + Mn	4.24×10^7	4.44×10^7	1950	1981	915	886
P + N	4.40×10^7	4.28×10^7	1989	1941	891	911
Macro-nutrients	4.26×10^7	4.42×10^7	1946	1985	880	922

SIGNIFICANT FACTORS ON FINAL DAY				
Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)				
(Fe + Mn)	*			
(P + N)		*		+
(MACRO)				

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 29

TESTING OF NUTRIENT GROUPS - TERTIARY EFFLUENT 25 Aug

EFFECT OF SPIKING

Nutrient Group Spiked	Cell Number (No/mℓ) With (a)	Cell Number (No/mℓ) Without (b)	Total Cell Volume ($mm^3/ℓ$) With (a)	Total Cell Volume ($mm^3/ℓ$) Without (b)	Dry Weight (mg/ℓ) With (a)	Dry Weight (mg/ℓ) Without (b)
Trace Metals	3.18×10^6	3.46×10^6	234	266	116	140
Fe + Mn	3.68×10^6	2.95×10^6	283	217	139	117
P + N	3.71×10^6	2.93×10^6	276	224	141	116
Macro-nutrients	3.07×10^6	3.56×10^6	223	277	120	136

SIGNIFICANT FACTORS ON FINAL DAY

Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)			*	-
(Fe + Mn)	**	**	*	+
(P + N)	**	**	*	+
(MACRO)	**	**		-

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 30

TESTING OF INDIVIDUAL NUTRIENTS - TERTIARY EFFLUENT 25 AUG.

EFFECT OF SPIKING

Added Single Nutrient	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
P	3.16×10^6	2.75×10^6	224	196	114	95
Fe	3.53×10^6	2.38×10^6	274	146	151	58
N	2.78×10^6	3.14×10^6	193	227	92	116
Mn	2.48×10^6	3.44×10^6	164	256	73	135

SIGNIFICANT FACTORS ON FINAL DAY

FACTOR	CELL NUMBER	CELL VOLUME	DRY WEIGHT	COMPARISON
P	**	**	**	+
Fe	**	**	**	+
N	**	**	**	-
Mn	**	**	**	-

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 31

CHEMICAL COMPOSITION OF ELECTRODIALYSIS EFFLUENT

Constituent	DATE OF SAMPLING 21 June 1971
Phosphorus mg P/ℓ	.271
Nitrate mg N/ℓ	.390
Nitrite mg N/ℓ	0.27
Kjeldahl Nitrogen mg N/ℓ	0.1[a]
N/P ratio	2.77
Iron µg/ℓ	1
Manganese µg/ℓ	2

[a]estimated value

Further, the reason for the negative effects of nitrogen on the algal growth are not clear from the results obtained and further evaluation of Electrodialysis Effluents should be made.

It should be noted that all biomass parameters are higher in individual nutrient spiking experiments. Examination of the detailed results in the appendix indicates that this is not caused by spiking alone, as control flasks (Nos 31 and 32) with nothing added are also much greater (mean cells/ml of 2.5×10^6 vs 4.5×10^5). It appears that the long storage period had considerable effect upon electrodialysis effluent.

Ground Percolated Effluent

Results of nutrient group spiking are shown in Table 34. Addition of the (P + N) group resulted in a highly significant increase in biomass for all growth parameters while the macronutrient addition increased only cell numbers. This data indicates that the (P + N) group is limiting in ground percolated effluent.

DISCUSSION - BATCH ALGAL ASSAYS

The two secondary effluent samples which were evaluated in this work exhibited very different chemical and algal growth characteristics. Characteristic of both samples was the fact that phosphate was not growth limiting (i.e. there was sufficient phosphate present); iron + manganese limited growth in one sample and nitrogen in the other. It should be recognized that the data here are based on only two samples and that broad conclusions cannot be drawn. Nevertheless, both samples were collected as 24 hour composite at a time when the activated sludge plant was performing in a normal manner. Based on these observations, it appears that more investigational work with a broad range of activated sludge effluents should be made.

TABLE 32

TESTING OF NUTRIENT GROUPS - ELECTRODIALYSIS EFFLUENT 21/22 June

	EFFECT OF SPIKING					
	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
Nutrient Group Spiked	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
Trace Metals	2.94×10^6	2.52×10^6	140	136	58	60
Fe + Mn	5.07×10^6	3.84×10^6	250	26	105	12
P + N	2.72×10^6	2.74×10^6	138	138	59	58
Macro-nutrients	2.22×10^6	3.24×10^6	103	173	40	77

SIGNIFICANT FACTORS ON FINAL DAY				
Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)				
(Fe + Mn)	**	**	**	+
(P + N)				
(MACRO)		*	*	-

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 33

TESTING OF INDIVIDUAL NUTRIENTS - ELECTRODIALYSIS EFFLUENT 21/22 June

	EFFECT OF SPIKING					
Added Single Nutrient	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
P	4.66×10^6	3.73×10^6	260	214	119	88
Fe	5.82×10^6	2.57×10^6	325	150	162	46
N	3.78×10^6	4.61×10^6	220	255	92	116
Mn	4.35×10^6	4.04×10^6	232	242	96	112

SIGNIFICANT FACTORS ON FINAL DAY

FACTOR	CELL NUMBER	CELL VOLUME	DRY WEIGHT	COMPARISON
P	**	**	**	+
Fe	**	**	**	+
N	**	**	**	-
Mn			**	-

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

TABLE 34

TESTING OF NUTRIENT GROUPS - GROUND PERCOLATED EFFLUENT 26 Feb.

	EFFECT OF SPIKING					
	Cell Number (No/mℓ)		Total Cell Volume ($mm^3/ℓ$)		Dry Weight (mg/ℓ)	
Nutrient Group Spiked	With (a)	Without (b)	With (a)	Without (b)	With (a)	Without (b)
Trace Metals	3.24×10^6	3.10×10^6	131	135	95	92
Fe + Mn	3.28×10^6	3.05×10^6	134	132	96	90
P + N	4.63×10^6	1.71×10^6	195	71	121	66
Macro-nutrients	3.34×10^6	2.99×10^6	136	130	97	89

SIGNIFICANT FACTORS ON FINAL DAY				
Factor	Cell Number	Cell Volume	Dry Weight	Comparison (d)
(Trace)				
(Fe + Mn)				
(P + N)	**	**	**	+
(MACRO)	**			+

a,b) Each value is the average of one-half of the 32 flasks in the factorial experiment. For example, the value at (a) is the average of the 16 flasks receiving the trace metal spike and the value at (b) is the average of the 16 flasks NOT receiving the trace metal spike.

(c) One star indicates that the addition of the group of nutrients resulted in a change in final biomass which was significant at the 95% level. Two stars indicate a corresponding significance at the 99% level.

(d) A positive comparison indicates that addition of the nutrient group increased the final biomass. A negative comparison indicates a decrease in final biomass when the nutrient group was added.

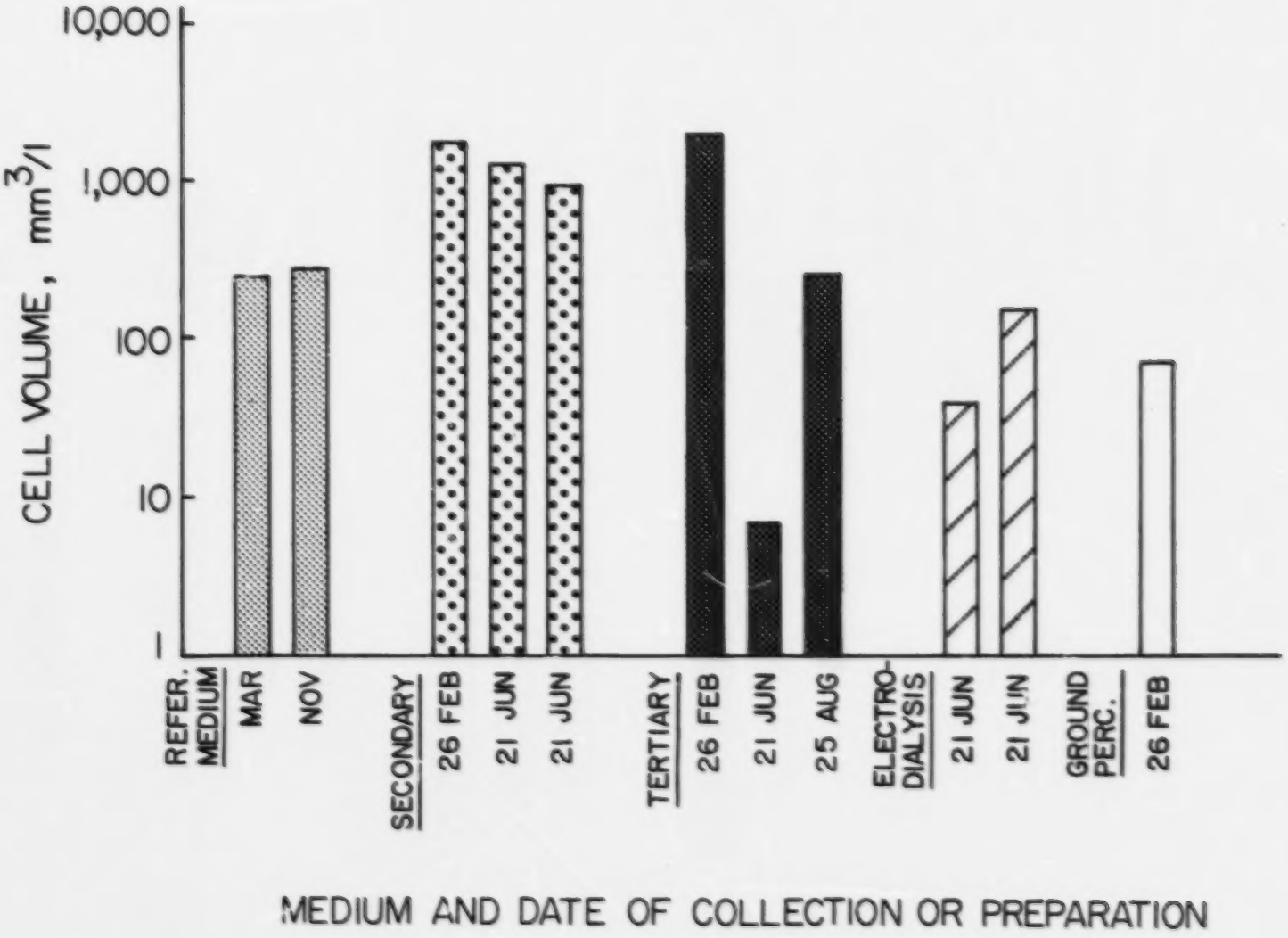

FIGURE 9. FINAL CELL VOLUME IN EFFLUENTS TESTED

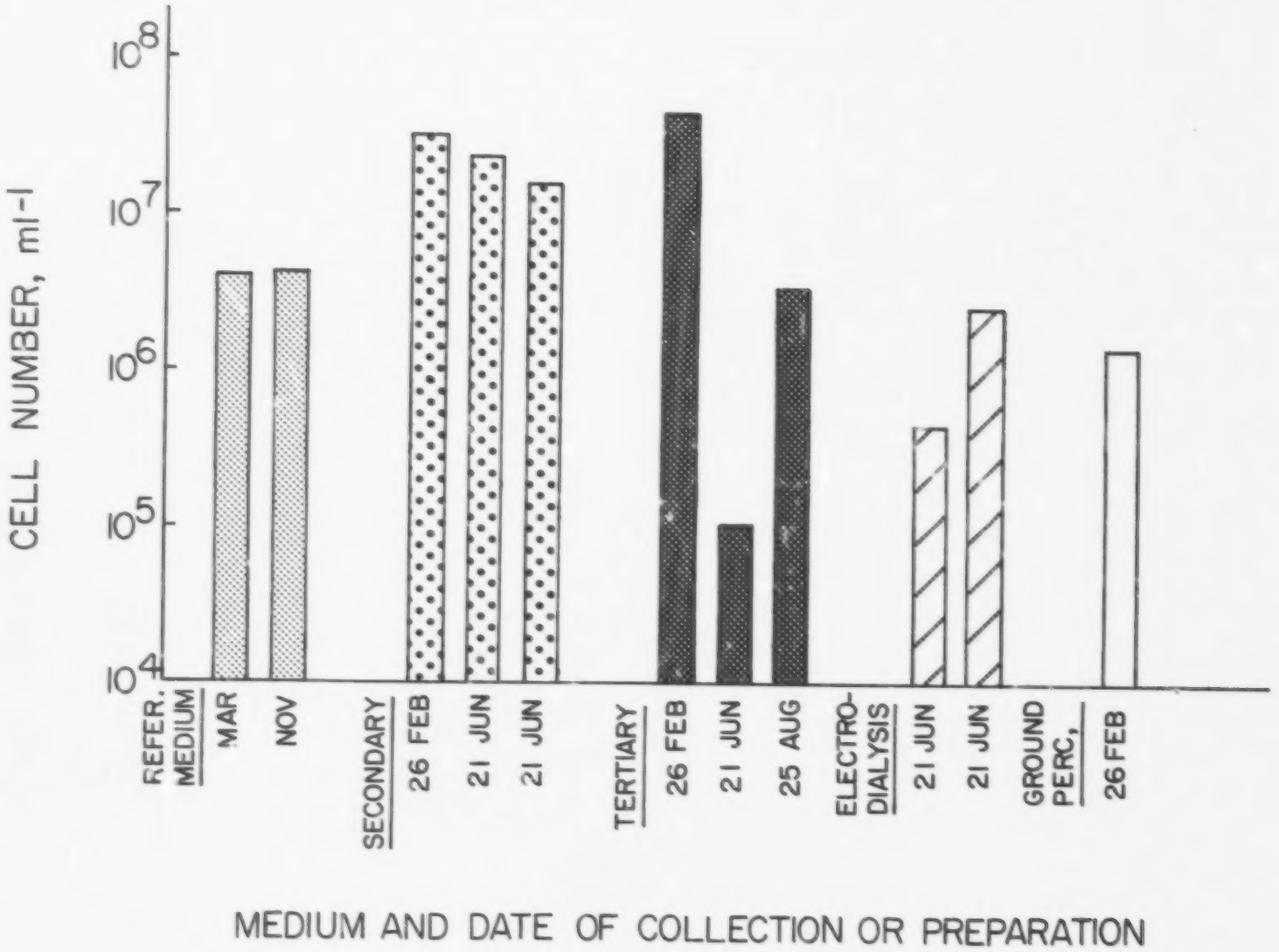

FIGURE 10. FINAL CELL NUMBER IN EFFLUENTS TESTED

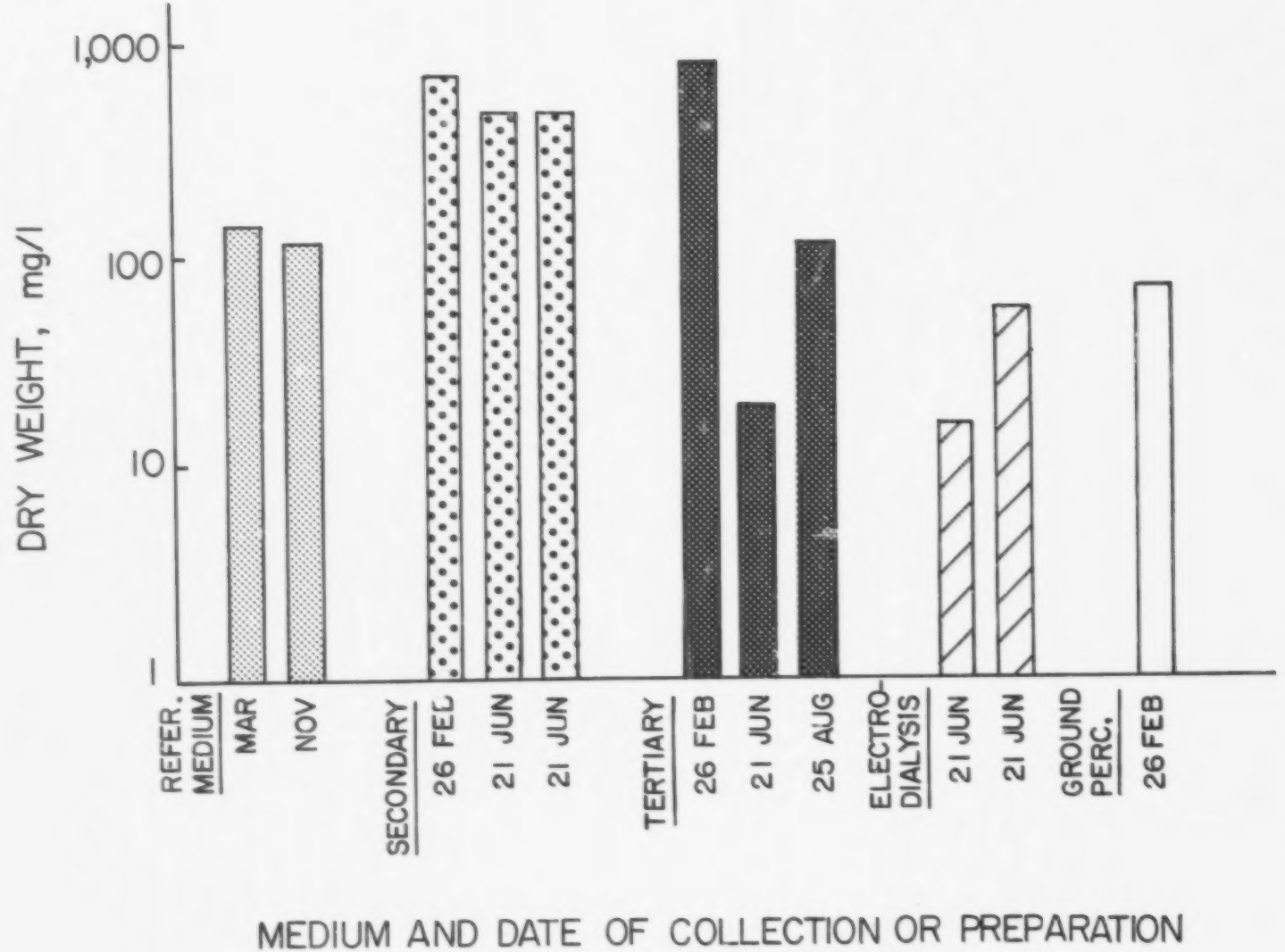

FIGURE 11. FINAL DRY WEIGHT IN EFFLUENTS TESTED

Of the two tertiary samples obtained, the one collected 25 August 1971 shows a much lower algal growth than the early sample collected 26 February 1971. These results are in agreement with the overall plant performance during the two periods of operation represented by the two samples. During the last period higher lime dosages were used and this resulted in better phosphate removal as measured by chemical analyses.

The higher lime dosages also resulted in better iron removal, and both iron and phosphate were limiting algal growth with iron being the most significant of the two as indicated by the results in Table 30.

At the same time nitrogen and manganese appeared to be suppressing growth. The reason for this effect is not understood at this time and further work should be conducted to verify this finding.

Evaluation of the electrodialysis effluent indicated that iron and phosphate were the growth limiting nutrients and that iron had the most significant effect. This is similar to the results obtained with the tertiary effluent.

The results of the batch assays are summarized on Figure 10, 11 and 12. These data are in general agreement with those obtained with the continuous culture units and signify to the relative improvement in effluent characteristics resulting from the tertiary treatment processes investigated at Santee.

CONTINUOUS CULTURE INVESTIGATIONS

Laboratory Investigations

Introduction

In order to evaluate a waste management program, or the quality of the effluents from such a program, a procedure which will provide data on different levels of algal growth at steady state may serve as a useful management tool. A continuous-flow laboratory culture can serve as such a tool because it simulates growth likely to occur in a body of water receiving a given effluent.

In the research described here, one liter chemostates were used (IV-2, VII-4, VII-5), and the results compared with the algal growths which occurred in the field in test ponds receiving the same effluents. With a residence time of 105 days, the laboratory chemostats required 99 days to reach steady state. This is a surprising length of time, in view of the consistency of the media and the conditions of culture. Steady state conditions were maintained for the recommended two residence times or 21 days. Measurement of cell number, mass and volume were performed on the effluents from the four chemostats on four or five occasions during this 20 day period.

The biomass expressed as dry weight (mg/l), for each of the three types of Santee effluents and the standard algal growth medium are shown in Figure 13. The results show:

1. The variation in biomass was insignificant over the 21 day period and that steady state conditions had, in fact, been achieved and maintained; and

2. The growth of the three effluents and the reference medium could be ranked in the following decending order, Secondary > Tertiary > NAMM > Electrodialysis.

Biomass can be expressed in various ways. Measurement of dry weight is useful in terms of energy values although in terms of practical application cell number and cell volumes may be the most useful parameters. Measurements of cell volume correlate best with optical density, what the eye actually sees, and this is the most likely assessment of algal growth, particularly by the nontechnical viewer. Average values for dry weight, (expressed as mg/l), cell number (expressed as number/l) and cell volume (expressed as mm^3/l) are given in Table 35.

In every case, the three effluents can be ranked in the descending sequence, secondary effluent > tertiary effluent > electrodialysis effluent. The values obtained with the reference medium place it between tertiary effluent and electrodialysis effluent, with values approximately half those for the tertiary effluent in every feature measured. The results are expressed graphically in Figure 13.

The major problems involved in these chemostat experiments were a consequence of flocculant material present, particularly in the tertiary effluent. The material appeared to be organic with possible nutrients chelated onto them. Filtration would have removed these nutrients so that they had to be retained in the chemostats despite the difficulties involved in using a Coulter Counter on fluids containing such flocculant material. It might well have been that the long time required for stability was consequence of slow equilibration with these chelated nutrients. Examination of the algae in the chemostats at the end of the experiment showed that after a period of nearly four months, the levels of contamination were not excessive. Bacterial browth was present in all the effluents and the reference medium, with the highest levels in the tertiary effluent. A small quantity of blue-green algal contaminant was also present in the chemostats with tertiary effluent.

Field Investigations

The objectives of the field assay investigations were to determine:

1. The total biomass which will develop in continuous culture, under field conditions, in the different types of effluent.

2. The species composition of the biomass in the effluents of different type.

3. The relative amounts of growth in the two ponds.

4. The comparison between absolute and relative amounts of growth under field conditions with the corresponding results determined in the laboratory continuous cultures.

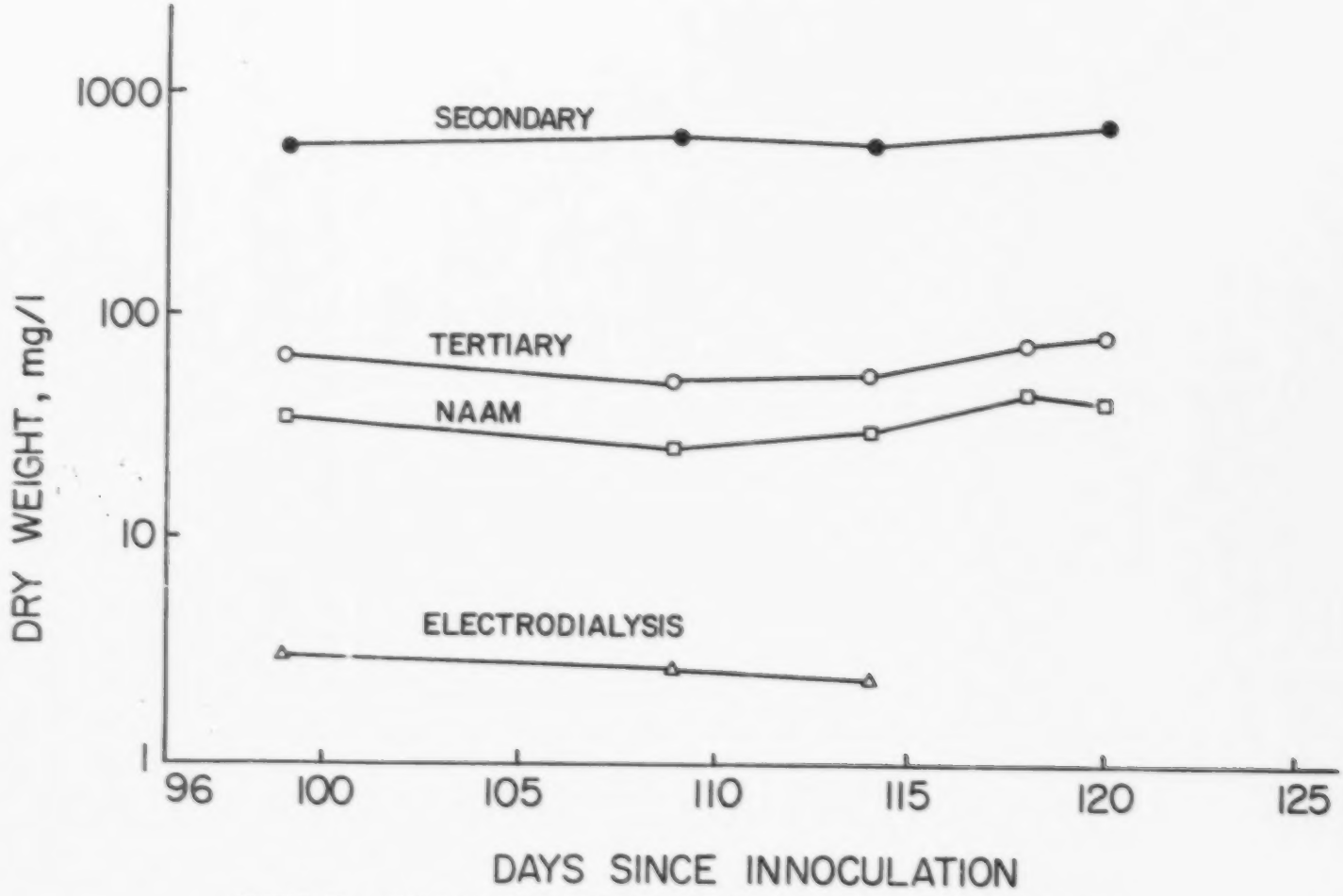

FIGURE 12. DRY WEIGHT IN CONTINUOUS FLOW CULTURES DURING STEADY STATE

TABLE 35

STEADY STATE BIOMASS CONCENTRATIONS IN CONTINUOUS CULTURES AT 10.5 DAYS RESIDENCE TIME

Biomass of chemostate continuous cultures of Santee secondary effluent, electrodialysis effluent, and reference medium expressed in terms of dry weight, cell number and total cell volume, using Selanastrum capricornutum as the test organism. These figures are mean values for the two chemostats established for each effluent and the reference medium.

Effluent or culture medium [a]	Dry Weight (mg/ℓ)	Cell Number per mℓ	Total cell volume ($mm^3/ℓ$)
Santee secondary effluent	580	144×10^5	1222
Santee tertiary effluent	63	7.50×10^5	110
Santee electrodialysis effluent	2.7	0.192×10^5	1.53
Reference medium	35.5	3.5×10^5	42

[a] All samples collected 21 June 1971

TABLE 36

BIOMASS IN STUDY PONDS

Biomass expressed in mg/l

DATE 1971	POND 2 Secondary Effluent	POND 3 Tertiary effluent
19 Mar	60	30
1 Apr	142	104
15 Apr	36	36
19 May	62	20
21 June	62	12
22 July	63	17
25 Aug	109	21
23 Sept	139	28
AVERAGE	77	33
Standard Deviations	46	28

Discussion

1. Algal biomass in ponds receiving secondary and tertiary effluents.

Considering the algal blooms present in the ponds receiving secondary and tertiary effluent, the values for the tertiary pond were (with one exception) lower than the values for secondary pond. Concurrent factors which varied significantly between the two ponds and might be influencing or reflecting these differences include:

1. Total phosphate was about 2 mg/l greater in the secondary pond than the tertiary.
2. Orthophosphate of the filtrate was also about 2 mg/l greater than the tertiary.
3. Alkalinity of the secondary was about 100 mg/l greater than the tertiary.
4. Ammonia filtrate of the secondary effluent was about 1.5 mg/l greater than the tertiary.
5. Suspended solids were about 35 mg/l greater than the tertiary.

Results

1. Estimates of total biomass in the ponds receiving secondary and tertiary effluents.

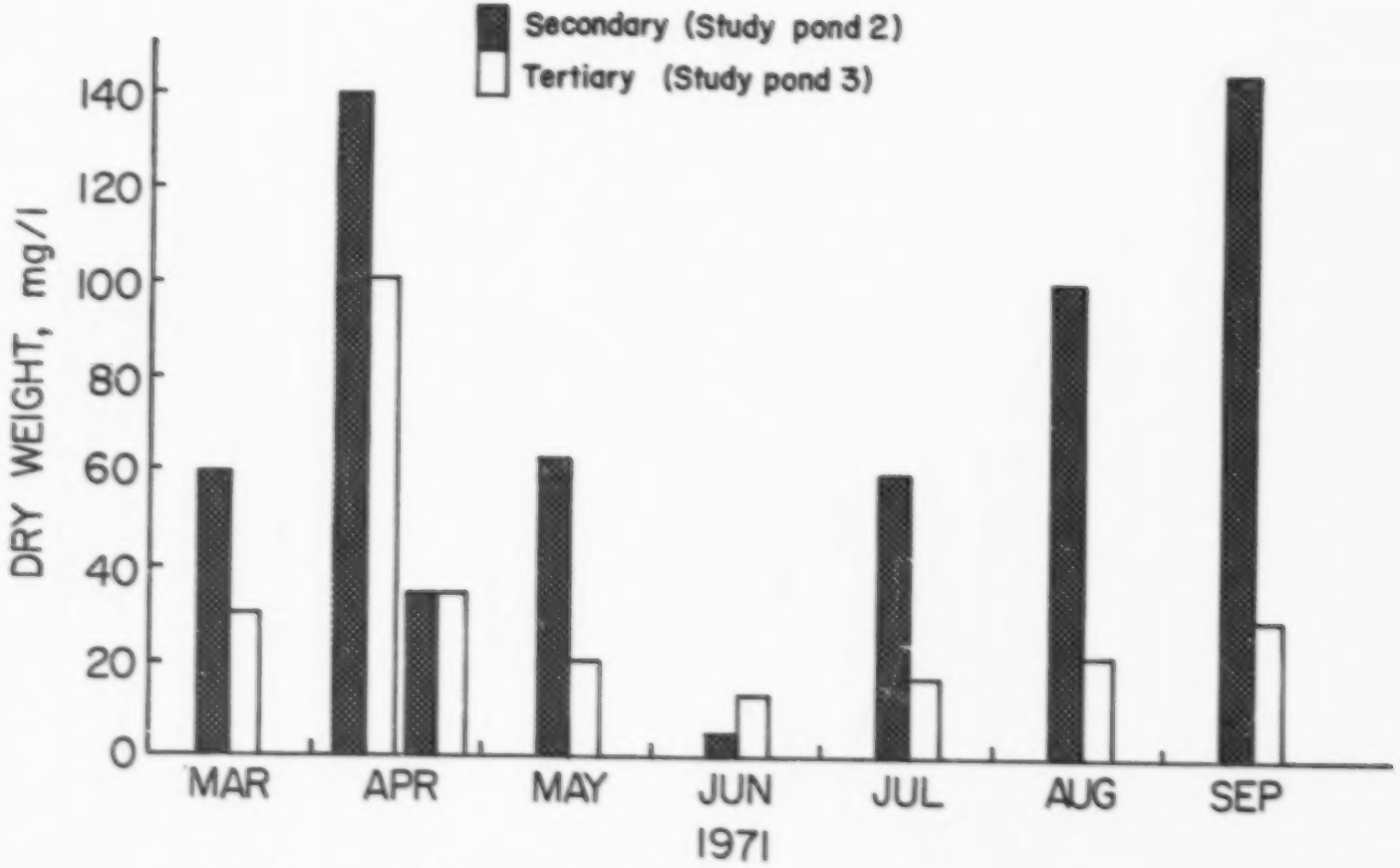

FIGURE 13. BIOMASS PRESENT IN STUDY PONDS

The algal biomass, expressed in terms of mg per liter, present in the ponds receiving secondary and tertiary effluents, during the period of investigation are shown in Table 36 and Figure 14.

2. Determinations of biomass by Secchi-disc measurements.

The depths at which the home-made Secchi-disc vanished are indicated in Table 37.

3. Identification of species and relative abundance.

Summaries of the major algal species in the ponds receiving secondary and tertiary effluents are given in Tables 38 and 39.

4. Chemical oxygen demand of secondary effluent was about 30 mg/l greater than that of the tertiary effluent, and

5. COD filtrate was about 20 mg/l greater than the tertiary.

TABLE 37

SECCHI DISK MEASUREMENTS IN STUDY PONDS

DATE	POND 2 (secondary)	POND 3 (tertiary)	% better clarity POND 2	POND 3
5 Aug '71	10 inches	22.5 inches		125%
20 Aug '71	11.5 "	14 "		21.7%
27 Aug '71	18 "	15 "	20%	
14 Sept '71	15 "	36 "		140%
AVERAGE	13.6 inches	21.9 inches		61%

At the same time -

1. pH of the two was about equal.
2. Dissolved oxygen was about equal; and
3. nitrate filtrate was equal.

However, nitrate filtrate of the secondary pond was slightly lower than the tertiary. The temperature during the entire study period climbed steadily from a minimum in February 1971 of 16°C to a maximum of about 25°C in August. Although the quality of tertiary effluent, as reflected in algal pond growth, had made a good recovery by mid-august, the secondary effluent did not show this rapid recovery and was continuing to deteriorate, in terms of the quantity of algal growth, as the project was terminated.

TABLE 38

COLOR OBSERVATIONS AND MAJOR ALGAL COMPONENTS - SECONDARY POND

DATE 1971	COLOR	ORGANISMS (abundant or 10^8)	ORGANISMS (frequent or 10^7)
26 Feb.	None recorded	naviculoid diatom	Cryptomonas Pedinomonas Sphaerellopsis (?)
19 Mar	None recorded	Euglena (naviculoid diatom abundant on rocks)	6-micron flagellate
1 Apr	Lower end darker; color not specified	Chlamydomonas Cryptomonas Eutroptia (?)	Euglena Scendesmus acuminatus Oscillatoria
15 Apr	Yellowish-green	None taken	None taken
19 May	Yellow-green	Chlamydomonas	Chlorogonium Oscillatoria
21 June	None recorded	Oscillatoria, 1.8×10^8/ℓ coccoid blue-green 3.78×10^8/ℓ	(Others, 10^6)
22 July	None recorded	coccoid green, 1.12×10^8/ℓ coccoid blue-green 4.20×10^8/ℓ Chlorogonium, 2.27×10^8/ℓ	Scenedesmus acuminatur, 2.25×10^7 Merismopedia, cells 4.28×10^7/ℓ flagellate, 1.87×10^7/ℓ Pedinomonas, 1.75×10^7/ℓ
25 Aug	Dark grassy green	Chlorogonium, 3.68×10^8/ℓ coccoid blue-green, 4.20×10^8/ℓ	coccoid green, 2.67×10^7/ℓ Merismopedia, cells 4.28×10^7/ℓ
23 Sept	Dark grassy green	Actinastrum, 1.08×10^8/ℓ coccoid blue-green, 3.28×10^8/ℓ Chlorogonium, 2.56×10^8/ℓ Chlamydomonas, 2.73 c 10^8/ℓ	coccoid green, 3.13×10^7/ℓ Oscillatoria, 1.25×10^7/ℓ

TABLE 39

COLOR OBSERVATIONS AND MAJOR ALGAL COMPONENTS - TERTIARY POND

DATE 1971	COLOR	ORGANISMS (abundant or 10^8)	ORGANISMS (frequent or 10^7)
26 Feb	None recorded	coccoid green Chlaydomonas	Chlorococcum (?) Euglena naviculoid diatom
19 Mar	None recorded	(2 flagellates and 2 green algae in 'occasional' amount)	12-micron flagellate
1 Apr	Lower end darker; color not specified	2-micron flagellate	3-micron coccoid green 5-micron coccoid green Chlaymydomonas
19 May	Brownish-green	(none abundant, others 'occasional to rare')	Ankistrodesmus Oscillatoria
21 June	None specified	coccoid blue-green, 1.6×10^9/ℓ Oscillatoria, 2.58×10^8/ℓ	Scenedesmus quadricauda, 1.0×10^7 Chlamydomonas, 1.0×10^7/ℓ chrysophyte flagellate,
22 July	None specified	(10^6 only)	coccoid green, 1.9×10^7/ℓ small green flagellate, 0.9×10^7/ℓ coccoid blue-green, 4.6×10^7/ℓ
25 Aug	Bluish - to light green	coccoid blue-green, 5.57×10^9/ℓ	(others 10^6)
23 Sept	Bluish-green	Actinastrum, 2.8×10^8/ℓ Pedlastrum, 2.8×10^8/ℓ	coccoid green, 5.50×10^7/ℓ Chlamydomonas, 1.0×10^7/ℓ coccoid blue-green, 7.25×10^7/ℓ Merismopedia, cells 2.5×10^7/ℓ

Clarity of the pond waters was measured with the Secchi-disc. The technique appears to be a simple method, highly suitable for assessing the amount of algal growth which is supported by the effluents in each of the study ponds. The major disadvantage is that particulate matter, which might be present, also influences the readings. This is particularly critical in any circumstances in which secondary effluent is likely to be used. The disc procedure measures total turbidity, i.e. of both particulate and biological origin, and the figures obtained correlate well with turbidity measurements taken at the ponds by the plant personnel, (Appendix A). Differences in disc measurements between secondary and tertiary effluents, as they occur in the ponds, are generally quite marked, with the tertiary pond registering greater clarity with one exception. Overall the tertiary pond average 61% greater clarity over the period of these measurements, taken during the hottest time of the year.

It is interesting to note that the tertiary pond, at the time when disc measurements were less than those of the secondary (27 August) was experiencing an abnormally high bloom of a coccoid blue-green alga. The bloom caused the pond to appear uniformly bluish-to light green. The pH at that time was on the decline to the point where it had just fallen below that of the secondary pond. Total phosphate and alkalinity were also low.

2. Floristic analysis of the algal growths in ponds receiving secondary and tertiary effluents.

The algal flora in a pond receiving a specifically treated and presumably well defined effluent might be expected to display uniform algal growth, particularly in a region such as Santee where climatic fluctuation is relatively limited. Unfortunately it was not possible to demonstrate evidence in support of this expectation as indicated by the summary of predominant species in Tables 38 and 39. The floristic changes, in terms of species composition and quantity, are as dramatic as any which have been found in any natural situation of an undefined nature (Talling, VII-6).

Although the study ponds did not support permanent populations of ducks or other waterfowl, transient visitors could be expected in almost any outside pond. Early morning surveys would be needed to confirm this. At Santee, bullfrogs were always apparent and more than once a large bittern was flushed from a small cluster of cattails which grew in the corner of one pond. By comparison with the nutrient status of the pond water, the enrichment contribution from these animals was undoubtedly negligible, but they act as a source of inoculation of the pond with new algal components carried by feet or feathers and in the alimentary tract. Atmospheric transportation of all manner of spores, cysts, and propagules can be expected. Although it had been anticipated with the sources of water available, that the flora of the ponds would be relatively uniform, this was not the case. The composition of the algal flora was as varied and completely unrelated between ponds as is usually the case with freshwater environments.

An effort was made to relate the color of the water in the different ponds with the species composition. These data are included in Tables 38 and 39. In general the secondary study pond consistently appeared more intensely green and more murky or turbid than the tertiary pond.

Color in the second pond varied from yellowish-green to dark grassy green. The most abundant organism, when yellow-green was noted, was Chlamydomonas, while another green flagellate (Chlorogonium), considerably larger than Chlamydomonas, was present in large numbers. However, the second flagellate was equalled in numbers by a blue-green filament which, by sheer size and pigmentation differences, apparently did not contribute much to pond hue. The presence of blue-green algae in less than bloom quantity is not easily detected. The figures can be misleading until the exceedingly small size of most blue-green algae is considered in comparison to the larger green flagellates, such as Chlorogonium. When two green and two blue-green organisms were present in almost equal numbers, the secondary pond appeared dark grassy green. It is interesting that on the same day (25 August) the tertiary pond a few feet away contained an excessive amount of tiny coccoid blue-green alga and appeared bluish to light green. Color is, therefore, not too relable a visual index of the constituents of the population supported by the pond. A correlation appears between turbidity and dry weight samples. It is suggested that Secchi-disc measurements be taken as a rapid, simple indication of standing crop. Unfortunately this was not appreciated until it was too late for meaningful incorporation in the present study. It is also suggested that water samples be taken more frequencly, preferably weekly, in order to monitor more precisely the changes in biotic composition.

3. Correlation between Chemical Parameters and Algal Biomass.

Correlation by visual consideration of the data failed to disclose any obvious relationship between the amount and kinds of organisms present and the chemical analyses of the study ponds (Appendix A).

An attempt was therefore made to relate the various chemical and physical parameters with the algal biomass over a series of periods throughout the duration of testing. A correlation coefficient was claculated for both secondary and tertiary effluents relating the algal biomass at the end of the test periods with the average values for each chemial and physical parameter during the period. The correlation coefficients are presented in Tables 40 and 41. The highest correlation coefficient with secondary effluent occurred with alkalinity and that was merely 0.52; no other value exceeded 0.50. For tertiary effluent, the correlation coefficients were slightly higher, but even so were not of any great significance. The highest value occurred for the correlation between nitrate filtrate and biomass and that was only .89. These data indicate that there is no acceptable correlation between the biomass and any chemical parameters for the two effluents when considered individually. However, the comparison between the growth in the two ponds substantiates the laboratory results.

TABLE 40 CORRELATION BETWEEN BIOMASS and PHYSICAL/CHEMICAL PARAMETERS - Secondary Effluent

Correlation, over 8 test periods, between the biomass on the last day of each period and the average values for various chemical and physical parameters for each period, in ponds receiving Santee secondary effluent.

Periods, indicated in days of the year	51-78	78-91	91-105	105-139	139-172	172-203	203-237	237-266	CORRELATION COEFFICIENT
Biomass, on last day of period (in mg/ℓ)	60	142	36	62	2	63	109	139	-
Total Phosphate	11.4	10.8	12.2	11.9	13.3	15.1	14.7	10.5†	.37
Orthophosphate	10.6	10.3	11.1	10.7	10.0	12.3	13.4	10.5†	.16
Alkalinity	175	148	120	149*	139	175	260	202	.52
Ammonium filtrate	4.2	2.5	4.0*	1.73†	1.32	3.38			.12
Suspended solids	33	37	46	42	41*	51	44*	37†	.32
COD	54.8	51.6	55.0	63.9*	45.8	54.3	53.5	64.4†	.42
COD filtrate	44.9	39.1	37.3	45.5	34.8	38.7			.30
pH	7.4	7.3	7.3	7.3	7.4*	7.4	7.1	7.3	.50
Nitrite filtrate	2.50	0.94	0.52	0.56*	0.48	0.54			.19
Nitrate filtrate	1.4	1.8	1.7	1.4	1.4	1.1			.42
Turbidity, Avg.	6.5	6.8*	8.3	10.9	7.3*	9.5	7.5	9.0	.05
Last day turbidity	4.0	8.5	13.0	7.0	7.0	10.0	9.0	14.0	.32
Number of points[1]	8	3	4	8	9	8	8	21	

[1] Number of points indicates number of days data taken in each period
* Number of data points less than indicated for particular period
† Outlier excluded from data

TABLE 41 CORRELATION BETWEEN BIOMASS and PHYSICAL/CHEMICAL PARAMETERS - Tertiary Effluent

Correlation, over 7 test periods, between the biomass on the last day of each period and the average values for various chemical and physical parameters for each period, in ponds receiving Santee tertiary effluent.

Period, indicated in days of the year	51-78	78-91	91-105	105-139	139-172	172-203	203-237	237-266	CORRELATION COEFFICIENT
Biomass, on last day of period (in mg/ℓ)	30	104	36	20	12	17	21	28	-
Total Phosphate	1.4	1.6*	1.8*	2.0*	1.0*	0.6		0.2*	.30
Orthophosphate	1.3*	1.7*	1.8*	1.9*	0.9*	0.3		0.1*	.40
Alkalinity	214	209	160	198*	155*	128			.52
Ammonium filtrate	3.6	1.6*	3.8*	1.4*	2.7*	4.1			.44
Suspended solids	17*	22*	27	24*	32*	27			.39
COD	34.6	32.1	29.4	29.5*	23.0	24.7			.53
COD filtrate	30.2	29.8*	29.0*	27.6*	21.1*				.53
pH	7.8	7.8	7.9	7.8	8.2	8.2			.52
Nitrite filtrate	0.15	0.38*	0.38*	0.23*	0.28*	0.26			.57
Nitrate filtrate	1.5	1.9*	1.4*	1.5*	1.4*	1.2*			.89
Turbidity, Avg.	3.4	2.1	2.5	2.8	2.8	2.8			.70
Last day turbidity	2.0	1.0	3.3	1.5	2.5	3.0			.61
Number of points[1]	14	7	8	17	18	12			

[1] Number of points indicates number of days data taken in each period
* Number of data points less than indicated for particular period
† Outlier excluded from data

SECTION VIII

REFERENCES

IV-1 Algal Assay Procedure - Bottle Test
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VII-6 Talling, F.G., Element of Chance in Pond Populations, Naturalist, pp 157-170 (1951).

SECTION IV

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1. Evaluation of Algal Assay Procedures-PAAP Batch Test by Steven Murray, Jan Scherfig, and Peter S. Dixon Journal WPCF, October 1971.

2. Algal Assay Procedure Bottle Test, National Eutrophication Research Program, Environmental Protection Agency, August 1971.

3. Evaluation of Materials for Use in Algal Culture Systems, by, Carol Justice, Steven Murray, Peter S. Dixon, Jan Scherfig, in Hydrobiologia, vol. 40-2, pp 215-221, 1972.

SECTION X

GLOSSARY

No special terms, abbreviations, or symbols used.

SECTION XI

APPENDICES

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TABLE A-1 CHEMICAL TESTS ON STUDY POND TWO, PART 1.

Date	Day of Year	Ortho-phosphate Filtrate mg/l-P	Total Phosphate mg/l-P	Ammonia Filtrate mg/l-N	Nitrite Filtrate mg/l-N	Nitrate Filtrate mg/l-N	Total Kjeldahl mg/l-N
Nov. 16, '70	320	0.96	0.98	9.38	0.76	0.50	13.2
Nov. 30	334	8.8	12.0	3.0	2.0	0.85	6.0
Dec. 7	341	---	---	---	---	---	---
Dec. 14	348	11.2	12.0	9.6	0.39	0.38	21.9
Dec. 28	362	5.80	6.60	5.85	2.1	0.35	9.3
Jan. 11, '71	11	6.80	7.20	3.68	3.0	0.80	10.5
Jan. 25	25	8.0	9.0	6.75	2.50	0.62	12.9
Feb. 8	39	3.6	5.0	5.5	2.0	1.0	14.1
Feb. 22	53	2.20	---	7.8	---	0.90	---
March 8	67	0.35	1.00	0.82	0.16	1.60	2.9
March 22	81	3.60	4.80	1.80	0.90	1.60	10.2
April 5	95	1.20	1.76	0.27	0.46	1.60	0.78
April 19	109	3.20	3.80	0.08	0.34	1.0	6.36
May 3	123	1.23	24.0	0.12	0.34	1.10	8.4
May 17	137	1.40	3.8	0.17	0.26	(.80	4.83
June 7	158	8.4	10.0	.92	.32	.90	8.19
June 21	172	3.40	5.80	2.90	0.44	3.5	8.19
July 12	193	16.0	16.4	0.08	0.19	2.20	6.03
July 26	207	1.76	4.60	0.22	0.13	0.60	4.52
Aug. 9	221	1.60	3.8	2.20	0.29	0.22	7.81

TABLE A-1 CHEMICAL TESTS ON STUDY POND TWO, PART 2.

Day of Year	pH	Suspended Solids mg/l	Turbidity JTU	COD mg/l	COD Filtrate mg/l	Color	Temp. °C	Dissolved Oxygen mg/l	Alkalinity mg/l
320	7.5	27	6.7	50.4	40.0	40	16	10.6	222.0
334	8.2	71	14	72.0	44.0	60	15	8.7	152.0
341	8.6	---	---	---	---	60	---	---	---
348	7.6	53	8	76.0	49.3	40	15	9.6	243.0
362	7.6	46	10	51.4	31.8	35	13	13.5	263.0
11	8.3	50	12	75.8	52.1	60	12	15.0+	224.0
25	7.9	49	12	86.6	51.5	70+	14	15+	260.0
39	8.7	96	12	100.0	51.0	70+	18	15+	217.0
53	7.8	26	6	58.9	---	40	16	8.3	278.0
67	8.2	34	17	19.7	10.7	30	17	13.4	109.0
81	7.8	87	22.0	67.9	40.0	25	18	13.4	169.0
95	9.5	133	19	73.6	42.0	40	20	13.8	67.9
109	9.4	71	22.5	61.6	37.6	30	16	12.6	84.0
123	9.6	96	20	76.8	49.7	40	18	10.8	2.6
137	9.1	63	22	65.7	32.4	30	19	8.6	83.0
158	8.0	89	8.0	40.4	33.6	25	23	9.0	189.0
172	8.2	52	17.0	48.8	42.8	25	22	8.1	221.5
193	9.2	86	27.0	69.5	38.4	40	24	7.8	117.2
207	9.4	105	27	79.2	44.6	40	23	7.6	84.0
221	9.1	90	22	76.4	56.6	40	24	8.9	118.8

TABLE A-2 CHEMICAL TESTS ON STUDY POND THREE, PART 1.

Date	Day of Year	Ortho-phosphate Filtrate mg/l-P	Total Phosphate mg/l-P	Ammonia Filtrate mg/l-N	Nitrite Filtrate mg/l-N	Nitrate Filtrate mg/l-N	Total Kjeldahl mg/l-N
Nov. 16, '70	320	0.98	1.0	4.70	0.46	0.65	5.7
Nov. 30	334	0.56	0.68	2.18	2.2	0.83	3.9
Dec. 7	341	---	---	---	---	---	---
Dec. 14	348	0.68	0.70	6.6	0.54	0.65	10.2
Dec. 28	362	0.10	0.46	5.40	0.66	0.37	6.0
Jan. 11, '71	11	0.34	0.39	3.0	0.54	0.40	9.6
Jan. 25	25	0.50	0.66	5.33	0.21	1.50	10.5
Feb. 8	39	0.34	0.70	2.9	0.49	1.2	3.01
Feb. 22	53	1.55	---	5.2	---	1.0	---
March 8	67	0.88	1.65	0.25	0.16	2.0	1.5
March 22	81	0.74	1.25	1.55	0.15	1.80	8.7
April 5	95	0.28	0.62	2.2	0.50	1.60	3.73
April 19	109	0.80	1.55	0.30	0.32	1.4	4.62
May 3	123	0.19	0.88	0.07	0.36	1.30	7.5
May 17	137	0.42	1.17	0.13	0.37	0.85	4.53
June 7	158	0.24	0.63	0.01	0.50	1.0	6.33
June 21	172	0.13	0.52	1.55	0.25	0.15	2.61
July 12	193	0.88	1.35	0.86	0.14	1.10	1.89
July 26	207	0.23	0.79	0.05	0.14	0.60	12.82
Aug. 9	221	0.05	0.40	0.42	0.28	0.50	3.72

TABLE A-2 CHEMICAL TESTS ON STUDY POND THREE, PART 2.

Day of Year	pH	Suspended Solids mg/l	Turbidity JTU	COD mg/l	COD Filtrate mg/l	Color	Temp. °C	Dissolved Oxygen mg/l	Alkalinity mg/l
320	8.6	12	2.2	30.0	27.6	20	15	11.2	223
334	9.7	25	3	30.4	28.0	20	15	8.5	128
341	9.3	---	---	---	---	25	---	---	---
348	9.2	35	5.8	47.9	38.2	25	15	10.2	185
362	9.3	41	12	41.0	28.5	25	12	11.8	207
11	9.4	27	3	43.9	34.2	25	12	15+	167
25	9.3	28	8	70.8	49.9	70+	14	15+	154
39	9.8	57	12	68.3	45.6	60	18	15+	165
53	8.1	27	11	60.7	---	45	16	6.6	212
67	9.4	30	13	32.8	28.3	50	16	17.1	197
81	8.6	78	15.0	59.1	40.9	45	19	14.6	188
95	10.0	79	12	78.4	44.0	35	19	14.8	28
109	9.7	67	12	52.0	27.0	25	16	10.6	77.7
123	10.0	82	15	69.1	41.2	40	18	10.2	76.0
137	9.8	50	13	69.0	39.1	30	19	8.8	74.5
158	10.0	95	13	66.0	38.0	45	24	15.0+	56.2
172	9.4	59	10	58.6	39.3	30	20	8.7	26.1
193	9.2	46	11	47.7	38.4	20	25	7.2	53.7
207	9.4	54	6	32.2	27.5	25	23	7.4	43.6
221	9.0	47	12	46.6	36.1	40	25	9.2	21.8

TABLE A-3 DISSOLVED OXYGEN TESTS ON THE STUDY PONDS.

Time & Date	Pond Two		Pond Three	
	D.O., mg/l	Temp., °C	D.O., mg/l	Temp., °C
1500, Aug. 19, 71	10.2	30	9.8	30
1700	10.1	27	9.6	27
1900	10.3	26	9.9	26
2100	8.0	26	7.1	26
2300	6.8	25.5	6.6	25.5
0100, Aug. 20, 71	6.5	25	5.9	25
0300	4.2	25	4.3	25
0500	2.8	24.5	3.3	24.5
0700	2.6	24.25	3.4	24.5
0900	5.7	25	5.3	25
1100	9.5	27.5	5.9	27
1300	9.7	30.5	8.5	29.5
1500	9.6	30	9.2	28